

A CHEMICAL INVESTIGATION
OF SOME
FLORIDA VOLATILE OILS

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INTRODUCTION

Florida with its varied and abundant vegetation offers many possibilities for study in the field of essential oils. In 1929 Werner (1) undertook a brief survey of some of the more readily available material. He pointed out at that time that, in addition to a large number of plants native to the State which had not been examined, there were also many others the constituents of which had been determined in other localities but which had not been studied from the standpoint of development under Florida conditions.

That farmers and other citizens of the state are interested in the commercial possibilities of volatile oil production is evidenced by the many inquiries received at the School of Pharmacy in Gainesville. Indeed four of the samples of oils examined in this study were sent in by B. W. Calvert, having been distilled by him from plants collected in the vicinity of Orlando, Florida. Another, oil of Illicium floridanum, became the object of interest through an observation by Dean H. H. Hume of the College of Agriculture that the leaves of this shrub were possessed of a rather attractive odor.

It has been well established that conditions of soil and climate exert an important influence on the yield and quality of volatile oils. That Florida possesses certain advantages in this connection is indicated from the demand in northern markets for Florida citrus fruits, garden truck, ferns, etc. It is also significant that in the case of Koellia mutica (Michx.) Britton,

one of the first of the native plants to be studied at the University of Florida, it was found (2) that the essential oil contained an exceptionally high percentage of pulegone, its chief constituent. Similarly with Mentha spicata Linne' experimental culture resulted in the production of an oil with a recorded carvone content (3) far in excess of that usually found in spearmint oils.

Another important advantage which Florida appears to offer to the commercial volatile oil producer is the possibility of two cuttings a year. For example where the Michigan and Indiana growers of spearmint harvest one crop annually, averaging 2 to 3 tons per acre, it has been found that two cuttings a year, totaling 6 to 7 tons per acre can be obtained in the University of Florida's Medicinal Plant Garden.

However in spite of the fact that it possesses an apparently favorable climate, some doubt was cast several years ago on the practicability of commercial production of mints, especially peppermint, in Florida. In 1927 A. F. Sievers, of the Bureau of Plant Industry, Washington, D. C., suggested that the School of Pharmacy, University of Florida, cooperate with the federal government in carrying out experimental tests with Mentha arvensis var. piperascens (Japanese peppermint). At about this time also root stocks of Mentha spicata L. and Mentha piperita L. were obtained and set out. The results of early spearmint cultivation were considered to be most promising and moderate success was achieved with Japanese peppermint but

with Mentha piperita L. the results were disappointing. In 1930 Dr. B. V. Christensen, Director of the School of Pharmacy, University of Florida, reported to the 4th Annual Symposium of the Genus Mentha (4) that a supposedly true type of Mentha piperita L. grown in the University's Medicinal Plant Garden the previous season had yielded an oil which assayed only 7.23% menthol and contained about 80% pulegone. He pointed out that the plant may have undergone dehybridization or a delayed reduction in the process of metabolism and expressed the hope that further study might explain the low menthol content of peppermints grown in the South.

Since several of the peppermint oils of the years 1929, 1930, 1931, and 1932 were still available, it was felt that a more detailed study of their composition and especially of the non-pulegone constituents should be undertaken. It is not improbable that these oils, by virtue of their abnormality, may disclose data which will later prove of value to others in their studies of the biogenesis of peppermint oil and the genetics of the genus Mentha.

This present investigation has therefore been undertaken with a double objective in view:

1. To determine the physical and chemical constants of several native oils and to identify such of their constituents as the circumstance of limited supply might permit.
2. To examine several oils distilled from Florida-grown Mentha piperita L. during the period 1929-32 and known to be low in

menthol content and in particular to seek to determine what other changes in composition may accompany failure to develop menthol and to record all other evidence of abnormality.

SOURCES OF MATERIAL

1. The following oils, included herein in Part I, were sent in for examination by B. W. Calvert of Orlando, Florida, having been distilled by him from plants collected in that region. The oils were received in the fall of 1936 and the quantities available for this investigation are indicated:

Oil of Pycnothymus rigidus20 cc.

Oil of Solidago rigida26 cc.

Oil of Erigeron canadensis28 cc.

Oil of Heterotheca subaxillaris. . 4 cc.

Mr. Calvert also supplied a specimen of the plant from which each of the oils was obtained and from this the identity was established in each instance by Mr. Erdman West, Botanist, Agricultural Experiment Station, University of Florida.

2. The oil of Illicium floridanum, also included in Part I, was distilled in March 1939 by the author from material made available through the kindness and cooperation of Dean H. H. Hume of the College of Agriculture, University of Florida. The leaves from which quantitative determinations were made were picked from shrubs on the grounds surrounding Dean Hume's residence in Gainesville. Later Dr. Hume arranged to have a larger quantity of fresh leaf picked from a stand known to him in the region of Florala in West Florida and transported to Gainesville. From this approximately 38 cc. of oil was obtained by steam distillation.

3. The several oils of peppermint, included in Part II, were

taken from storage in the Department of Pharmacognosy, University of Florida. The particulars in Table 1. with respect to their origin were obtained from departmental records and from data recorded by Hiner.* The quantities available for this investigation are also indicated.

Table 1.

Sample No.**	Origin of Root Stock.	Distilled.	Green Herb.	Yield (in cc.)	Yield Per Acre	Quantity Available
2.	Washington	June 25/29	525 lbs.	1330	36.03 lbs.	950 cc.
3.	"	July 7, 8/30	1065 "	1620	25.4 "	625 "
4.	"	Oct. 28/30	400 "	675	11.86 "	430 "
5.	"	Aug. 7/31	55 "	115	-----	70 "
6.	Oregon	Aug. 12/32	316 "	630	-----	250 "
7.	Washington	Sept. 26/29	682 "	1230	33.15 "	750 "

The reference to Washington as the source of original root stock is believed to be to Washington, D. C. and not to the State of Washington. Hiner in his preliminary study of some of these oils puts aside the possibility of mistaken identity as follows(5):

"Plants from the garden were identified here as having the characteristics of Mentha piperita, but in order to eliminate any doubt specimens were sent for verification to A. F. Sievers, Bureau of Plant Industry, Washington, D. C., from whom the original root stock was obtained. Reports from Messrs. Sievers and Lowman of that office stated that no mistake was probable since the specimens from Florida compared exactly with the

* M.S. Thesis, University of Florida, 1931.

** Sample No. 1. of this series of oils was a Japanese peppermint oil.

original stock growing in the Government garden and producing oils of relatively high menthol content". Certainly no evidence can be found in Pharmacognosy departmental records to indicate that the reference is to the State of Washington.

DISCUSSION OF METHODS OF EXAMINATION AND PROCEDURES USED.

Standard works such as Gildemeister and Hoffmann (6) and Parry (7) contain a wealth of detailed information regarding methods of examination of volatile oils. However, new methods and procedures are constantly being devised and the literature has been searched with a view to selecting those which might be of value in the conduct of this investigation. In particular, in view of the small amounts of some of the samples available, the application of micro and semi-micro methods was of special interest.

A short discussion of some of the methods used is included at this time in order to avoid too frequent reference to procedures and overlapping in the recording of the experimental work. The literature with respect to methods which have a general bearing on the investigation is also reviewed.

1. Determination of Physical Constants:

The methods employed in determining the physical constants require very little comment since more or less standard methods were followed. Refractive index readings were made with an Abbe-Zeiss refractometer and optical rotations were determined with a half shade, Duboscq Precision Model polarimeter.

The specific gravities, when the quantity of material was very small, were obtained by means of specially prepared weighing bottles made from flat-bottomed glass ampoules. These could be quickly filled by means of a hypodermic syringe and were

particularly advantageous when room temperature was several degrees higher than that at which the determinations were to be made. The degree of accuracy was found to be quite satisfactory when checked against a Sprengel tube.

For the determination of melting-points a Fisher-Johns melting-point apparatus was found to be very useful. The results obtained with this small electrical heating unit were checked in several instances with the ordinary method and showed good general agreement. In some cases the yield of derivative was so small that its melting-point could not have been reported by any of the usual means. Special characteristics of the fusion, such as sintering of crystals, presence of odd-shaped and unmelted crystals, etc., are readily discernible with this instrument.

A method of determining boiling-points which will not waste material becomes an important consideration in carrying out fractional distillations with small quantities of oil under reduced pressure. This problem was studied experimentally and the conclusion was reached that when carried out under standardized conditions the method described by Shriner and Fuson (8) could be relied upon to give a reasonable approximation of the true boiling-point. In the present work this method was even more satisfactory than the method described by Kamm (9), which requires more material.

2. Determination of Free and Combined Alcohol:

Determinations of acid and ester values were carried out

by the usual straight-forward methods. The employment of ester value after acetylation, as an indirect means of quantitatively determining free alcohols, is however, worthy of some comment. Of the oils concerned in this present study, four do not appear to have been reported on previously. One, oil of Erigeron canadensis, has been stated to contain the tertiary alcohol terpineol. The peppermint oils, of course, should contain the secondary alcohol menthol. It has been recognized for some time that, while the reaction between acetic anhydride and an alcohol is quantitative in the cases of primary and secondary alcohols, results with linalool, terpineol and tertiary alcohols generally are much less satisfactory. These, when boiled with the anhydride are partially decomposed by splitting out water. This, therefore, should be borne in mind when an oil of unknown composition is being investigated. Boulez (10), in 1907, attempted to prevent the decomposition of tertiary alcohols by diluting them with an indifferent solvent and acetylating the mixture. He found that good results were obtained when the proportions were 20 per cent of substance and 80 per cent of xylene. Schimmel and Company (11) report that under these conditions the maximum of ester is reached after boiling 5 to 7 hours. They found 99.8 % with reference to 100 parts terpineol known to be present. Recently this matter of determining free alcohols has received considerable study. Delaby and Bruegnot (12) report that the methods of Boulez (xylene) and of Glichitch (cold formylation) give accurate results for total alcohols

but are not selective. These authors also state that the rapid catalytic processes of Fernandez and Mingo and of Sabetay include all alcohols but are deficient as regards tertiary; and that all of these methods, as well as that of Zerevitinoff and methoxyacetylation, are vitiated by the presence of phenols, aldehydes and amines. Free primary and secondary alcohols are sometimes determined by conversion into acid phthalic esters, particularly by the method of Radcliffe and Chadderton (13) which has been studied by Glichitch and Naves (14) and shown to be unaffected by the presence of tertiary alcohols, aldehydes, phenols and esters. Verley and Bölsing (15) recommended a method of acetylation in the presence of pyridine which has recently been examined by Delaby and Bruegnot (12) and modified and applied with some success by them. Under their conditions it is contended that primary alcohols, primary amines, and phenols are acetylated quantitatively in $\frac{1}{2}$ to 1 hour, secondary alcohols almost quantitatively in 1 hour, while tertiary alcohols and aldehydes scarcely react at all.

For the oils of unknown composition and for oil of Erigeron canadensis it was decided that the method of Boulez might be employed appropriately in this investigation, inasmuch as several writers have reported it to give reliable results for total alcohols when tertiary alcohols are present. In carrying out this method 3 to 5 cc. of oil was placed in a small weighing bottle and accurately weighed after which exactly four times its weight of xylene was added. This mixture was carefully

transferred to an acetylation flask and acetylated in the usual manner, using 6 cc. of acetic anhydride and 0.6 gm. anhydrous sodium acetate. The boiling was continued for six hours after which the mixture was removed to a separatory funnel and the flask rinsed out with three successive 5 cc. portions of warm water, adding the rinsings to the funnel. After drawing off the aqueous layer, the remaining acetylated mixture was washed successively with portions of a mixture of equal parts solution of sodium carbonate and water until the washings showed an alkaline reaction with 2 drops of solution of phenolphthalein. The acetylated oil was then well dried over anhydrous sodium sulfate and 5 to 8 gm. taken for saponification.

Schimmel and Company (11) have pointed out an error in Boulez's original calculation of his results in that he failed to take into account the changed weight relationship of oil to xylene after acetylation. It will be realized at once that where the alcohol present is known this error may be corrected within reasonable limits. It is felt, however, that the error in any case will be considerably less when a tertiary alcohol is involved than if the ordinary method of acetylation is used. An examination of the results in determining the free alcohol content of oil of Erigeron canadensis furnishes a good case in point. Determined by the ordinary method and by the Boulez method the ester values after acetylation (uncorrected) were 45.15 and 75.05 respectively, showing a decided difference in favor of the latter method. The value 75.05 is found

to correspond to 16.82 % of free alcohol (calculated as terpineol) and when these figures are corrected they become 72.44 and 16.61 % respectively. Due to the difficulty sometimes experienced in judging accurate endpoints in titrating saponification mixtures of acetylated oils (the darkened color of the oil somewhat obscures the color change), this difference at least in the case of oils of low alcohol content, is regarded as well within the limit of experimental error.

When the experimental work in this investigation was in its final stages Brignall (15) published the results of a study in which a new rapid method for determining menthol in oil of peppermint is described. The application of this method in this present study has been restricted to the peppermint oils and it will be discussed in more detail when the experimental results are recorded in Part II. However, it is referred to at this time since it appears to be applicable without variation for the analysis of free primary and secondary alcohols, regardless of content, in any essential oil. The utilization of an acetylating mixture of n-butyl ether and acetic anhydride is believed to permit a more selective esterification of alcohols than the U.S.P. XI method and since saponification is eliminated more satisfactory results are obtained with those alcohols whose esters hydrolyze slowly or with difficulty. The method is rapid and the presence of a relatively large bulk of water in the titration mixture enables the endpoint to be judged accurately.

3. Detection and Separation of Phenols, Aldehydes and Ketones:

Phenols and aldehydes usually interfere with the quantitative determination of alcohols. It therefore becomes important in examining oils of unknown composition to test for the presence of such compounds and, if they are found, they should be removed before undertaking the determination of esters and alcohols. Qualitative tests for phenols are well known and the usual method of removal is by absorption in aqueous alkali. With respect to reliable qualitative tests for detecting the presence of aldehydes, however, a few comments seem to be justified.

It has been noted from the literature that many investigators appear to use the classical methods, such as with Tollen's and Schiff's reagents. These reagents are very sensitive to aldehydes and would appear to be capable of detecting the same in traces far beyond the limits of possible separation. For example, oils of Solidago rigida and Erigeron canadensis gave positive reactions with Schiff's reagent, the latter oil quite definitely so, yet in neither case could a satisfactory addition product be obtained by ordinary means. In 1929 Rodionow and Korolew (17) suggested a new qualitative reaction for aldehydes in which intensely yellow crystalline oxazolones are produced by the condensation of the aldehyde with hippuric acid. These oxazolones are difficultly soluble but form a red solution with sulfuric acid, being precipitated unchanged upon dilution. It is also worthy of note that ketones do not appear to give this reaction. The test is carried out by mixing equimolecular

portions of anhydrous sodium acetate and hippuric acid and then, after addition of a few drops of acetic anhydride and the substance under examination, heating the mixture on a water-bath for 5 to 10 minutes. A portion of the mixture is then poured into concentrated sulfuric acid when the presence of an aldehyde is shown by the appearance of a blood-red color followed by the formation of a yellow precipitate after dilution with water. Of the oils examined only oil of Erigeron canadensis, which gave the strongest Schiff reaction of any, gave an appreciable yellow precipitate. This was significantly less than the precipitate obtained when a like amount of Oil of Cinnamon was tested as a control. Since this test appears to be less sensitive than when Tollen's or Schiff's reagents are used, it may find some application in detecting aldehydes present in measurable amounts in volatile oils.

Worthy of particular note is the essentially micro-chemical method described by Fisher and Moor (18) for the detection of aldehydes and ketones in essential oils. The vapor from the oil is brought into contact with the reagent (either phenylhydrazine or semicarbazide in aqueous or acetic acid solution) a drop of which is suspended on the under side of a cover-slip, which may be placed directly over the mouth of the container. In this manner crystalline addition compounds may be obtained which, after careful washing and drying on the cover-slip, can be used for melting-point determinations. Fisher and Moor used Köfler's modification of Klein's micro

melting-point apparatus. In this investigation the small Fisher-Johns heating unit previously referred to was found to be quite satisfactory. This technique has the advantage of conserving material and seems to present many possibilities in the qualitative examination of volatile oils. It was of considerable assistance in the present investigation.

The two most important reagents used in the separation of aldehydes and ketones from the volatile oils are undoubtedly sodium bisulfite and semicarbazide hydrochloride. In view of their wide application in laboratory and technical practice it would hardly seem necessary to comment on them here. Yet surprisingly little information is available in standard reference works concerning the inner mechanisms and peculiarities of these reactions. For example, in Gildemeister and Hoffmann (6), considered by many the classical reference work in the field of volatile oils, one finds the bare statement that ketones which will not combine with acid sulfites, such as menthone, fenchone, and carvone, are converted into oximes or semicarbazones. No attempt is made to explain the fact that menthone does not give an addition product with sodium bisulfite while pulegone on the other hand does.

The work of Raschig (19) and of Raschig and Prahl (20), (21) on the constitution of aldehyde and ketone bisulfites has resulted in a much clearer understanding of the mechanism of the reaction. Dodge (22) has summarized this and other recent work and embodied the findings with respect to addition compounds between bisulfites and carbonyl compounds in general

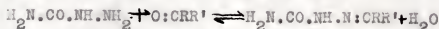
into the following classification:

1. O compounds, in which the bisulfite group NaHSO_3 appears to be attached to the carbonyl group. These are generally only slightly soluble in water and bisulfite solution, are more or less dissociated in solution, and are readily decomposed by acids and alkali carbonates.
2. Alpha compounds, in which the addition appears to be on an unsaturated linkage adjacent to the carbonyl group. These are generally formed in alkaline solution, are readily soluble, and are decomposed by alkaline hydroxides but not readily by carbonates. In addition to aldehydes and ketones, alpha compounds may be obtained from unsaturated acids, such as acrylic, cinnamic, and fumaric acids, and from unsaturated lactones such as coumarin and its homologs.
3. Omega compounds, in which the addition is at some position not adjacent to the COH or COR group. These compounds are soluble and are not decomposed by acids or alkalies under ordinary conditions so the original compound cannot be regenerated. Conditions which appear to favor the formation of omega compounds are stated to be dilute solution and especially absence of free SO_2 .

It is entirely possible for sulfonates of the three types to occur together in the same molecule and inspection of the formula should show what types are possible. However, actual formation of the compounds depends on conditions which are at present mostly unknown, although some facts are coming

to light. For instance Dodge (23) records that carvone dissolves as alpha compound in 1 mole of Na_2SO_3 and may be recovered with the original rotation. On the other hand with two moles NaHSO_3 it dissolves as alpha-omega disulfonate and cannot be recovered. Thus it appears that the use of normal sulfite or of bisulfite to form addition compounds with aldehydes or ketones, whether for the purpose of analysis or for separation, should not be undertaken without some previous consideration being given to such factors as the chemical structure of the carbonyl compound and the possibility of addition compounds resulting from other constituents which may also be present.

Semicarbazide, like other hydrazine derivatives, will condense with compounds containing a carbonyl group with elimination of water to give semicarbazones:



These compounds are valuable from a qualitative standpoint because they usually crystallize well and are not very soluble. Furthermore, since they can be hydrolyzed by aqueous acids to the original carbonyl compound they are of value in the separation and purification of aldehydes and ketones. It will be noted that the above reaction is reversible and recently a number of studies have been made regarding equilibria and rates in the formation of semicarbazones. In one of these (24) it has been noted that there is no relationship between the rate of formation and the ease of hydrolysis; a point of interest in connection with the regeneration of aldehydes and

ketones from volatile oils. Worthy of note also is the fact, reported by several investigators, that semicarbazide acting on unsaturated ketones under certain conditions gives, in addition to the ordinary semicarbazones, semicarbazide semicarbazones, i.e. products of the addition of one molecule of semicarbazide to the semicarbazone at the double bond. It is also known,* but not often stated in reference texts, that solutions of semicarbazide decompose slowly on keeping or on heating to form the amide of hydrazine dicarboxylic acid ($\text{H}_2\text{N.CO.NH.NH.CO.NH}_2$) and this substance, which is sparingly soluble, melts at $245-6^\circ\text{C}$, and has often been mistaken for a semicarbazone.

The procedure which in this investigation was found to give the most satisfactory results is as follows: 1 cc. of the fraction was dissolved in 10 cc. of alcohol and sufficient water added to just render the solution turbid. This turbidity was just removed by the addition of more alcohol and then 1 gm. of semicarbazide hydrochloride and 0.73 gm. of anhydrous sodium acetate were added. The mixture was placed in a bath of boiling water, from which the flame had been removed, shaken occasionally until the solids dissolved and allowed to stand until the bath had cooled. It was then allowed to remain in the refrigerator, removing the separated crystals at intervals, and diluting with water after each filtration, until no further separation took place. In many instances a deposition of a small amount of the insoluble hydrazodicarbonamide took place immediately after cooling and this

* Sidgwick's Organic Chemistry of Nitrogen (1937) p. 287.

was usually removed at once. The technique of Hugh, Kon, and Linstead (25) was found to be of great value in freeing the semicarbazone from occluded material. It is of interest to note in this connection that many workers and some authors record the melting-point of pulegone semicarbazone at $167-9^{\circ}\text{C}$. By this procedure no great difficulty was experienced in raising the melting-point to $173-4^{\circ}\text{C}$ (Heilbron* records 174°C).

4. Identification of Volatile Acids:

Many different methods have been employed for separating and identifying the volatile acids which are present in essential oils both in the free and the combined states. Among those which may be mentioned are fractional distillation, fractional precipitation or crystallization of their salts, and fractional extraction with solvents. Most workers in the field of volatile oils seem to prefer the separation as silver salts. During the course of this investigation Dunkley (26), who was studying the application of the Duclaux method to the determination of small amounts of unknown volatile acids in dairy products, suggested its possible utilization in the examination of volatile oils.

The method originally proposed by Duclaux in 1874 has been studied and elaborated by Gillespie and Walters (27). It is an indirect method based upon the behavior of acids during distillation from dilute aqueous solution. The procedure consists in distilling a dilute solution of volatile acids made up to a definite volume (110 cc.), collecting the

* Heilbron's Dictionary of Organic Compounds (Oxford University Press), 1934.

distillate in 10 equal fractions (10 cc. each) and titrating the acid in each fraction. It has been determined that the acid distilling in the first 10 cc. is a definite percentage of whatever quantity is taken for distillation, and so for the first 20 cc., etc., and furthermore that the same quantity of acid will be collected in any given fraction whether or not other acids are distilling at the same time. The percentages are not dependent upon the quantity of acid taken for distillation, but are constant for the given acid. The above mentioned authors (27) found by the application of the method to known mixtures that mixtures of two or three acids may be quantitatively analyzed without too great error, but that errors are too large in general for mixtures of four acids. When four or more acids are present they recommend fractionation of the mixture into mixtures containing only three acids in significant quantity before applying the Duclaux method.

This method, it would seem, has a definite place in volatile oil chemistry. It is true that a certain amount of technique must be acquired before results can be relied upon and that calculations are somewhat involved. However, the method itself is reasonably rapid and there is the advantage that, after its completion, the acids are still available in total amount for other tests of a confirmatory nature. It is also worth noting that the errors of the Duclaux method are not considered to be proportional to the quantity of acid present. Dunkley

(26) after considerable experience with the method attempted determinations if as little as the equivalent 5 cc. of N/10 acid was available. Good results can be anticipated with the equivalent of 15 to 30 cc.

P A R T I

Chapter 1.

OIL OF PYCNOTHYMUS RIGIDUS

Pycnothymus rigidus (Bart.) is placed by Small (28) in the family Lamiaceae. It is stated by this same authority to be a small shrub of rather wide-spread occurrence in southern peninsular Florida and to be commonly used in making tea. A search of the literature failed to disclose evidence of any previous investigation of the volatile oil from this plant.

EXPERIMENTAL

The sample, 20 cc., was greenish-yellow in color, possessed a pleasantly aromatic and strongly mint-like taste, while the odor also carried a very strong suggestion of mint.

The constants of the oil were determined to be as follows: d_{4}^{20} 0.9323; n_D^{20} 1.4681; α_D^{20} -4.14°; A.V. 1.75; E.V. 135.51; E.V. after acetylation 272.17; the boiling range extended from 160° to 220°C. with the greater portion distilling between 214-215°C.

A small portion of the oil when examined by the sodium fusion method proved to be free from nitrogen and sulfur.

Phenols: The oil showed a negative phenol reaction with solution of ferric chloride and likewise when treated with solution of potassium hydroxide in a Babcock tube no evidence

of absorption was detected.

Aldehydes and Ketones: A slight positive reaction was obtained with Tollen's and Schiff's reagents. When a drop of semicarbazide reagent was placed on a cover-slip and inverted over the mouth of the bottle a trace of crystal formation could be detected. This, in the absence of a more positive aldehyde reaction, was interpreted as suggesting the likely presence of a ketone, which was later confirmed through a bisulfite addition product.

Examination of Impure Fractions: Since the quantity of sample available did not permit a proper fractionation, an effort was made to utilize the distillates obtained while determining the boiling range of the oil for various identification tests.

1. The small portion, less than 1 cc. (from 5 cc. of oil), which passed over at about 160°C. was found to have a n_{20}^D of 1.4638. No crystalline nitrosochloride could be obtained.

2. A second portion, about 3.5 cc., which passed over between 207 - 220°C. was found to have a n_{20}^D of 1.4668.

(a) Preparation of hydrogen phthalate esters: To 2 cc. of this fraction 1.25 gm. of phthalic anhydride was added and the mixture heated in a test-tube, under reflux, for 10 hours at 115°C. After cooling, the mixture was added to solution of sodium carbonate in a separatory funnel and allowed to stand to permit complete hydrolysis of the excess anhydride. This was then shaken out with 15, 10, and 10 cc. portions of ether

to remove unesterfied oil and neutral phthalic esters. The aqueous layer, which showed a dark brown immiscible layer at the top, was next acidified with dilute HCl until no further precipitation occurred and the hydrogen phthalate esters shaken out with chloroform. The chloroform was removed, first by evaporation on a water-bath and finally under diminished pressure, whereupon a considerable amount of a sticky, brown-colored residue remained. This residue proved to be readily soluble in pentane but all efforts to obtain it in crystalline form by means of this and other solvents were unsuccessful.

(b) Preparation of a phenyl urethane: To the remaining 1 cc. of the fraction 0.5 cc. of phenyl isocyanate was added and the mixture was warmed on a water-bath for about 10 minutes and then cooled in a refrigerator. Small needle-shaped crystals soon began to form and after standing over night these were removed and dried between filter papers. The crystals were then subjected repeatedly to boiling pentane and upon evaporation of the solvent a small quantity of crystalline residue remained, melting-point $240-242^{\circ}\text{C}$. The original crystals, after extraction with pentane, also melted $240-242^{\circ}\text{C}$. so it was concluded that both residues consisted only of diphenyl urea.

Identification of the Ketone: 3.5 cc. of the sample, dissolved in 50% alcohol, was heated on a water-bath with 2.5 gm. of sodium bisulfite dissolved in 5 cc. water. The mixture was then set aside in a refrigerator for 48 hours, after which time an appreciable layer of crystals was found on one side of the

test tube. The whole contents of the tube was transferred to a separatory funnel and the unchanged oil removed by shaking out with ether. The ketone was regenerated by treatment with sodium carbonate and recovered by steam distillation. The first distillate was of milky appearance and possessed a strong pulegone-like odor but, upon standing, no noticeable separation took place. The weak distillate was cohobated and added to the first distillate and the whole tested successively by the hanging-drop method with semicarbazide, hydroxylamine and phenylhydrazine reagents. The results of this treatment were somewhat indefinite. The globule, in the case of phenylhydrazine reagent, when examined under a microscope showed distinct evidence of an oily addition product, but no crystals. With semicarbazide an amorphous solid mass remained on the cover-slip after washing with water. This appeared to melt rather indefinitely at 84-85.5°C.; and with hydroxylamine a distinctly crystalline residue was obtained, melting-point 115-117°C.

The whole distillate was then treated with semicarbazide reagent, warmed on a water-bath for 10 minutes and placed in a refrigerator. After several days, no semicarbazone having separated, the mixture was steam distilled and again set aside in the refrigerator. Once again no separation took place but after freezing the mixture to a solid mass with dry ice a slight yellow residue remained on the bottom of the container after liquefaction. This was filtered out and dried and a melting-point of 240-243°C. observed. This residue was therefore

judged to be hydrazodicarbonamide (m.p. 245-246°C.).

DISCUSSION OF RESULTS

The constants, as determined for this oil seem to indicate that it consists principally of an alcohol, apparently almost equally divided between the free state and the ester. Assuming the composition $C_{10}H_{18}O$, the ester values before and after acetylation calculate out to the equivalents of 47.23 % ester and 47.02 % free alcohol. The alcohol with which the physical constants seem to come into closest agreement is thujyl alcohol (29) (b.p. 206 to 207°C., d. 0.9229 to 0.9230, n_D^{20} 1.4625 to 1.4758). Simonsen (30) describes this alcohol as a colorless, somewhat viscid oil, with an odor somewhat resembling that of carvo-menthol. This remark with respect to odor can be said to be quite applicable to both the characteristic odor of the oil and of the 207-220°C. fraction. It will be observed that the reported value of n_D^{20} 1.4668 of the latter falls nicely within the range reported for thujyl alcohol. No phenyl urethanes are reported in the literature for this alcohol nor could one be obtained with this fraction. As a result of failure to obtain a crystalline hydrogen phthalate ester positive identity was not established. Shortage of material prevented a further attempt in this direction by oxidation to thujone.

It was thought that the very small fraction of the sample which distilled over at about 160°C. might constitute a terpene fraction. Both the boiling-point and the refractive index are not far removed from those reported for thujene and for sabinene, neither of which appear to form crystalline derivatives readily.

This latter fact may be of some significance in that the small portion available for test failed to yield a nitrosochloride.

The identity of the ketone present in this oil cannot be said to have been established. It is apparently present in very small amount and the odor carried a strong suggestion of pulegone. Pulegone oxime is stated to melt at 120-121°C. and in this case the microchemical evidence of an oxime, m.p. 115-117°C., was fairly good but no semicarbazone corresponding to pulegone semicarbazone could be obtained.

Chapter 2.

OIL OF SOLIDAGO RIDIDA

Solidago rigida L. is a member of the Compositae, or goldenrod family, which is widely distributed over North America, being represented by about seventy-five species. According to Small (31) this herb has a stem 5-20 dm. in length and is found in swampy woodlands and thickets, various provinces, Ga. to Mo., Ont. and Me.

Several oils of the genus *Solidago* have been reported in the literature. In 1893, Oberhauser (32) made a chemical examination of the flowering plant of S. rugosa Mill. and reported the presence of 0.996 % of a volatile oil. The flowers and leaves were distilled separately with water. Both oils had an odor resembling oil of origanum and gave evidence by their reactions with bromine and iodine of containing large proportions of terpene. Schimmel and Company (33) reported in 1894 that they had obtained a yield of 0.63 % of a volatile oil from S. canadensis L. The oil had a light yellow color and very agreeable, sweetish-aromatic odor. The constituents were later reported (34) as pinene 85 %, phellandrene, dipentene, limonene, borneol 9.2 %, bornyl acetate 3.4 %, and cadinene. In 1906, Schimmel and Company (35) also reported receiving from America a sample of oil derived from S. nemoralis. This oil had a bright olive-green color and a peculiar odor, reminding somewhat of cypress.

In 1914, Miller and Eskew (36) using the fresh herb (root excluded) prepared an oil from S. nemoralis, by steam distillation, which they stated had color and odor similar to the sample described by Schimmel and Company. It will be seen, however, (Table 2.) that the constants as described by them are considerably different from those reported by Schimmel and Company. With respect to constituents, Miller and Eskew reported 0.6 % phenol; aldehydes and ketones absent; α - pinene as the chief constituent; salicylic acid and acetic acid; and at least one alcohol, occurring both in the free state and combined as acetate. The alcohol was suspected to be borneol but could not be definitely proven.

In 1915, Miller and Moseley (37) prepared and examined two oils of the genus Solidago, namely, oil of S. rugosa and oil of S. odora. It was found that α - pinene comprised the greater part of oil of S. rugosa but that limonene and probably β - pinene were also present as was a small amount of an alcohol in both the free and combined states. The proportion of this latter was calculated as being equivalent to 1.67 % borneol and 1.47 % bornyl acetate. S. odora was official in the United States Pharmacopolia from 1820 to 1870 and the oil from this plant was known, in a small way, in commerce for many years. This golden-rod oil, as it was known, was in all probability a nondescript oil distilled from a mixture of weeds without any attempt at selection. Miller and Moseley obtained their oil, on the other hand, in yields in excess of 1 % by steam distillation of fresh

material, carefully examined and known to be free from other plants. The color of the oil was slightly yellowish, the odor somewhat like that of anise and somewhat suggestive of safrol, the taste warm and sweetish but noticeably less sweet than anise. Regarding the chemical constituents of this oil, they report phenols absent; aldehydes and ketones absent; terpenes, mostly pinene (10-15 %); esters about 3 %, calculated as bornyl acetate; alcohols about 3 %, calculated as borneol; methyl chavicol about 75 %.

The constants as reported for the above mentioned oils are summarized in Table 2.

Table 2.

Oil	<i>S. canadensis</i>	<i>S. nemoralis</i>	<i>S. odora</i>	<i>S. rugosa</i>		
Observer	(33)	(35)	(36)	(37)	(32)	(37)
d_{15}°	0.8590	0.8799	0.8532 _{15°}	0.9310 _{15°}	0.8486* 0.8502†	0.8620 _{15°}
α_D	-11° 10'	-23° 10'	-16.17°	+13.72°		-12.8°
n_D			1.47397	1.5065		1.4813
S.V.		14.4	5.6	7.9		4.22
S.V. after acetylation		38.2	9.4			10.97
Soluble in		7 vols. of alc. 90%	4 vols. of alc. 90%			

* from flowers.

† from leaves.

EXPERIMENTAL

The sample (about 26 cc.) was almost water-white and both taste and odor were somewhat remindful of oil of pine. The constants were determined to be as follows: d_{4}^{20} 0.8500; α_D^{20} +38.15°; n_D^{20} 1.4798; A.V. 0.39; E.V. 8.68; E.V. after acetylation 160.15. When a small sample (3 cc.) was carefully heated in an oil-bath, the greater portion appeared to distil over at about 165°C. leaving a residue of less than 0.5 cc.

When examined by the sodium fusion method, the sample was found to be free from nitrogen and sulfur.

Phenols: With solution of ferric chloride and likewise with solution of potassium hydroxide there was no evidence of the presence of phenols.

Aldehydes and Ketones: The oil reacted negatively with Tollen's reagent and to the test of Rodionow and Korolew but there was a slight coloration with Schiff's reagent. When tested with semicarbazide by the micro-method the result was negative.

Examination of Impure Fractions: Tests for the purpose of identifying the constituents of this oil were of necessity restricted to the distillate and residue from the boiling range determination.

1. The distillate (2.5 cc.) which as previously stated passed over at about 165°C. was found to have an n_D^{20} of 1.4820. A nitrosochloride was prepared (m.p. 96.5 - 99°C.) but efforts to prepare a nitrolpiperidide from this produced only an oily separation and no crystals.

2. The residue was utilized for the preparation of a phenylurethane. Some difficulty was again experienced with diphenyl urea but eventually a small amount of a crystalline residue was obtained which melted 136-140°C. The melting point of bornyl phenylurethane is given (6) as 138-139°C.

DISCUSSION OF RESULTS

The results obtained by the rather restricted chemical examination of this oil must be regarded as not in good agreement with the evidence furnished by the physical constants. The low density (0.8500) is at once suggestive of high terpene content and yet an ester value after acetylation of 160.15 was found. Since the odor of the oil was suggestive of borneol which as was noted above, also has been reported in other solidago oils, the ester values were calculated out and found to correspond to 3.04 % bornyl acetate and 47.35 % free borneol. The melting point of the phenylurethane (136-140°C.) lends strength to the conclusion that borneol may be present as one of the constituents. However, since the presence of 47.35 % of borneol can hardly be stated to be substantiated by the observation that the greater portion of the oil distilled over at 165°C., the possible additional presence of some other lower boiling-point alcohol must be considered.

No definite conclusions can be stated with respect to the nature of the terpene, or terpenes, present. In appearance the nitrosochloride bore a close resemblance to a similar compound

prepared from pure α -pinene and it is possible that the presence of other terpenes might have prevented a higher melting-point than the 99°C. observed and hindered the separation of a definite nitrol-piperidide. It may also be said that the boiling-point and refractive index of this distillate point to the likely presence of limonene, or dipentene, or both. The refractive index 1.4820 is considerably higher than would be expected for a nearly pure sample of α -pinene.

Upon comparing the observed constants with those reported for other solidago oils (see Table 2.), the most striking contrast is offered by the high ester value after acetylation (160.15) noted for oil of Solidago rigida.

Chapter 3.

OIL OF ERIGERON CANADENSIS

Erigeron canadensis L. is a very common weed, sometimes known as fleabane, horseweed, or butterweed, and often found growing in mint fields. It appears to have wide distribution. Upon distillation the fresh herb yields 0.33 to 0.66 per cent of oil (38).

In 1854, Procter (39) reported that oil of erigeron had been introduced recently into medical practice by the eclectic physicians. He described it as, "of a light straw color; very limpid; peculiar aromatic odor, not unpleasant and somewhat analagous to hemlock; peculiar taste, mild and not very pungent". He remarked further, "that it distils over per se (boiling range 155-185°C.), unchanged which is probably oxidized oil". Considerably later (in 1881) Vigier and Cloëz (40) described the oil as of yellow color, weedy odor, acrid burning taste and stated that in contact with air it oxidizes rapidly, producing a red-brown deposit. With dry hydrogen chloride these authors prepared a compound of composition $C_{10}H_{16}.2HCl$ from the oil, which was verified by Beilstein and Wiegand (41).

In 1884, Wallach (42) obtained a tetrabromide from oil of erigeron which was identified as limonene tetrabromide (m.p. 104-105°C.).

Todd (43) carried out an extensive botanical and chemical study of Erigeron canadensis and reported the results of an examination of eight samples of the oil (see Table 3.). Using

a sample supplied by Todd, Power (44) determined some of the constants and reported a carbon-hydrogen content equivalent to $C_{10}H_{16}$.

In 1893, Meissner (45) pointed out that the boiling-points given by Vigier and Cloëz (175-176°C.) and by Beilstein and Wiegand (176°C.) and by Power (176°C.) correspond well with the boiling point of pure limonene (175-176°C.). The dihydrochloride obtained by Vigier and Cloëz was held to be no absolute proof, in itself, for limonene since it might also have been obtained from pinene, terpineol, terpin-hydrate, etc. Wallach's tetrabromide was, however, accepted as limonene tetrabromide. Meissner further records that the 176°C. fraction from Todd's experiments was subsequently examined in the laboratory of Dr. E. Kremers. The nitrosochloride was prepared, according to the method of Wallach, and converted into the benzylamine base, which, after washing with alcohol and drying, melted at 89-91°C. (after one recrystallization this rose to 93°C.) The α_D for this fraction was determined to be +87.90°. From these findings it was concluded that the terpene definitely was d-limonene. Using a sample of oil obtained from Fritzsche Bros., New York, Meissner confirmed this observation that the chief constituent is d-limonene. Even those fractions which possessed a somewhat higher or lower boiling point evidently consisted almost entirely of this hydrocarbon, the boiling point of which was apparently slightly raised or depressed by other substances. No evidence was found to support the possible

presence of pinene and the principal constituent of the oil besides limonene was considered to be a high boiling substance, probably aldehyde-like in character, since it so readily decomposed and polymerized. Later, in 1895, Hunkel (46) prepared a nitrosochloride from Meissner's 205-206°C. fraction the nitrol-piperidide of which was observed to melt at 159-160°C. This was believed to be a derivative of terpineol and it was therefore concluded that the second constituent of oil of erigeron is d-l terpineol. Schimmel and Company (47) carried out a distillation study of Erigeron canadensis collected in the neighborhood of Miltitz (Germany) and more recently Kazakevich and Sobolevskaja (48), in 1928, reported on an oil obtained from herb collected in Saratov (Soviet Union) and Gaponenkov (49), in 1933, upon one produced in Turkestan. The last mentioned oil was stated to contain mainly limonene and probably dipentene, terpineol and methylethylacetic acid.

The constants reported for the oils mentioned above as well as those of some other observers have been summarized in Table 3.

Table 3.

Observer	(39)	(40)	(43)	(47)	(48)	(49)
Yield				0.264%	0.4%	0.189- 1.726
d_{15}°	0.845	0.848(10)	0.856- 0.870	0.8720* 0.8836†	0.8685	0.8732- 0.8764
α_D		+16° 15'	-23° to -60°	53° 56* 50° 4' †	80° 55'	64° 55' to -55° 5'
n_D^{20}				1.4992* 1.5062†	1.4790	1.4894
A.V.				0.3 * 0.3 †	2.79	0.00- 0.018
E.V.				63.5 * 70.9 †	39.09	48.14- 53.5
E.V. after acetylation				70.3 * 81.9 †	68.18	51.99- 64.3
Boiling Range C.	155-185	175-177	172-175			
Soluble in				5.5 vol* 4.0 " † alc. (90%)		

* plants just beginning to flower

† later stage of development

EXPERIMENTAL

The oil was light amber in color with an odor not unlike dill, although somewhat suggestive of lemon. It possessed a taste remindful of caraway. The sample (28 cc.) at the time

of examination showed distinct evidence of partial oxidation. The constants were determined to be as follows: d_{4}^{20} 0.8642; $\alpha_D^{20} + 71.70$; n_D^{20} 1.4809; A.V. 1.94; E.V. 17.12; E.V. after acetylation 75.05. When the boiling range was determined, on a 5 cc. sample, a small fraction, less than 1 cc., distilled over at about 145°C ., while about 3 cc. passed over in the region of 170°C . leaving a dark brown residue.

Tested by the method of sodium fusion the oil was found to be free from nitrogen and sulfur.

Phenols: There was no reaction with ferric chloride and no absorption when treated with solution of potassium hydroxide.

Aldehydes and Ketones: This oil gave a positive reaction with Tollen's and Schiff's reagents and with the test of Rodionow and Korolew.

An effort was made to obtain an addition product with bisulfite. No crystals separated after standing for several days and subsequent treatment with alkali and steam distillation failed to produce any separation.

Upon treating 1 cc. of the sample with semicarbazide a small yield of crystalline material (m.p. $149-152.5^{\circ}\text{C}$.) was obtained.

Examination of Impure Fractions:

1. The small portion which distilled over at about 145°C . yielded a semi-crystalline nitrosochloride (m.p. $96-98^{\circ}\text{C}$.) but no crystalline nitrol-piperidide could be prepared from this compound.
2. The portion which distilled over $170-175^{\circ}\text{C}$. was found to

have an n_D^{20} 1.4751. A nitrosochloride was prepared which, after one recrystallization, melted at 99.5°C . In converting this compound to the corresponding nitrol-piperidide, difficulty was experienced in obtaining a crystalline product. Finally after dissolving the residue in cold alcohol and allowing this to evaporate spontaneously, almost to dryness, a semi-crystalline mass was obtained (m.p. $73-75^\circ\text{C}$.). Further recrystallization failed to raise the melting-point above 78°C . at the time, but after standing for about two weeks a well diluted alcoholic solution produced crystals, m.p. $92-93^\circ\text{C}$.

3. The brown liquid residue was treated for the preparation of a nitrosochloride by Wallach's (50) method. No crystalline product could be separated.

DISCUSSION OF RESULTS

The physical constants found for this sample are in fairly close agreement with those reported in the literature and particularly with those of recent observers (48) and (49). Different samples of the oil apparently vary widely in acid value, as might be anticipated from the fact that it oxidizes readily (39) (40). Mention has been made above of the visible evidence of oxidation in the sample at the time of examination and it will also be noted that the ester value (17.12) is lower than those reported by others. Schimmel and Company report a particularly high ester value (70.9). In respect to ester value after acetylation, it is not known by what method the

values shown in Table 3. were obtained and, since terpineol has been identified in at least one sample (46) of oil of erigeron this is a point of considerable importance. Although identification of this alcohol could not be established in the oil under examination, the wide gap between the ester value of 45.15, as determined by the ordinary method and 75.05, by the Boulez method, does point to a tertiary alcohol. When calculated out, the values found in this instance are equivalent to 16.82 % terpineol and 5.94 % terpineol acetate.

At least one investigator (45) has called attention to the likely presence of an aldehyde in oil of erigeron but no record could be found of successful separation and identification of the same. In this sample the qualitative evidence of aldehyde was unmistakable but it is believed that the proportion present is very small. The amount of yellow oxazolone formed by condensation of the aldehyde and hippuric acid appeared very slight in comparison with that from oil of cinnamon under the same conditions. Although an addition compound with bisulfite could not be obtained what was believed to be a trace of a semicarbazone was separated and the melting-point observed at 149-152.5°C. This does not appear to check with any of the recorded aldehydes but, in view of the fact that a distinct odor of citral was noted in one of the saponification value residues, the statement of Gildemeister and Hoffmann(51), that mixtures of the semicarbazones of citral a and citral b show melting-points between 130 and 171°C., is of interest.

The evidence furnished by the boiling range (170-175°C.) of the main fraction and the nitrol-piperidide (m.p. 92-93°C.) obtained from this fraction is fairly definite for the presence of limonene. The impure nitrosochlorides from this fraction and from the small portion which appeared to pass over at a lower temperature melted at approximately the same temperatures. The presence of at least one other unidentified substance in these first two fractions might account in part for the difficulties experienced in obtaining crystalline nitrol-piperidides.

Chapter 4.

OIL OF HETEROTHECA SUBAXILLARIS

Heterotheca subaxillaris (Lam.), according to Small's (52) classification, is a member of the thistle family (Carduaceae). It is a small herb whose habitat is pine-lands, sand-dunes and waste places, various provinces, Fla. to Tex., Ariz., Kans., and Del. No reference could be found of any previous investigation of the volatile oil from this source.

EXPERIMENTAL

The oil possessed a yellow color and a strong pine-like odor and taste.

With this sample (4 cc.) the examination was confined mainly to such constants as could be determined. The following results were obtained: d_{4}^{20} 0.8877; n_D^{20} 1.4931; A.V. 0.48; E.V. 26.13; E.V. after acetylation 160.91; boiling-point (micro-method) about 200°C.

When tested by sodium fusion, the oil was found to be free from nitrogen and sulfur.

DISCUSSION OF RESULTS

The odor of the oil suggested the possible presence of borneol and this is at least not contradicted by such factors as could be determined. For example the oil appeared to boil

at about 200°C. whereas the fraction of 205-212°C. is the one usually taken for the separation of borneol.

Calculating the ester values obtained in terms of $C_{10}H_{18}O$ it is found that they are equivalent to 9.15 % combined and 42.28 % free alcohol. No material remained for the preparation of a phenylurethane.

Chapter 5.

OIL OF ILLICIMUM FLORIDANUM

Illicium floridanum Ellis, family Magnoliaceae, is stated by Small (53) to be a shrub 2-3 m. tall found in swamps and low hammocks, coastal plain, N. Fla. to La. and N. Ala. Various common names have been applied to it among which may be mentioned Southern Star-Anise, Florida Stink-bush, Purple Anise, Poison Bay.

The genus *Illicium* is important because of the commercial use of the volatile oil which is obtained from the fruit of Illicium verum. This well-known species and I. religiosum are natives of China while two others, I. floridanum and I. parviflorum are reportedly found in the southern states of North America. I. parviflorum has also been reported as occurring in other regions, notably Madagascar (54) and Anam (55) but I. floridanum would appear to be restricted to this continent.

The official, or Chinese star-anise (I. verum Hooker) has been extensively investigated. The Japanese star-anise (I. religiosum Siebold), because of the fact that the fruit resembles that of the true star-anise so closely that it is often mistaken for it or sold as an adulterant, has also received considerable botanical and chemical attention. S. Y. Chen (56) has compiled an extensive bibliography in this latter connection.

In 1901, Schlotterbeck and Eckler (57) stated that, in contrast to I. verum and I. religiosum, the American species

have been practically neglected, a remark which would be almost equally true today. In 1885, Maisch (58) made a botanical study of I. floridanum and published a short description of the histological characters of the root, stem, leaf, capsule, and seed along with several plates of drawings. Later Schlotterbeck and Eckler carried out a more detailed study, which was likewise botanical in nature. To date no complete phytochemical study of I. floridanum appears to have been made nor have the constants of the volatile oil been reported. Recently Foote (59) examined a volatile oil obtained from the leaves of I. parviflorum Michx. by steam distillation and reported a high safrol content (over 90 %) with but traces of acids and esters. The United States Dispensatory makes reference to an oil from this same species under the title of oil of anise bark, it being stated that the bark yields fully $3\frac{1}{2}$ % of a light yellow oil, the odor of which resembles safrol and having the constants: $d_{15}^{\circ} 0.969$; $\alpha_D -0^{\circ} 46'$; $n_D^{15} 1.5251$. It is further stated to contain a small quantity of ordinary anethol, but to consist principally of the isomeric methylchavicol.

Because of the close approach of some of the constants of the oil of I. floridanum to those of oil of I. religiosum, a few of the findings in connection with the latter oil might well be mentioned at this time. In 1932, Schimmel and Company (60) reported that the oil from the leaves and fruit of I. religiosum, differs widely from that of the Chinese star-anise. It was reported that Eykman had detected in it a terpene,

eugenol, and safrol, while Tardy, working with an oil extracted from the fruit with petroleum ether and subsequently purified by distillation in vacuo, noted an α_D of $-1.50'$ and the presence of eugenol. From the fraction of $170-177^\circ\text{C}$. he identified cineol, either anethol or methylchavicol was believed to have been present in the fraction $220-230^\circ\text{C}$., and evidence of safrol was found in the fraction $230-235^\circ\text{C}$. In 1909, Schimmel and Company (61) reported constants for the oil (see Table 4.) and at this time it was stated that the oil differs from the oil of the Chinese star-anise chiefly in the fact that, unlike the latter, it contains scarcely any anethol, while its principal constituent is safrol, which the Chinese oil contains only in traces. The Japanese oil, therefore, congeals only at about -18°C ., with separation of safrol, and when subjected to fractional distillation under a pressure of 3.5 mm. cineol was identified in one fraction and the presence of linalool in another fraction was suspected. In 1926, K. K. Chen (62) reported the constants for an oil prepared by steam distillation from the dry carpels (see Table 4.) and later, in 1929, S. Y. Chen (56) carried out a very complete phytochemical study of I. religiosum. The constants obtained by S. Y. Chen for a volatile oil, prepared by steam distillation from the oily material separated from an alcoholic extract of the finely powdered fruit, are also given in Table 4.

Table 4.

OIL OF ILLICIUM RELIGIOSUM

Observer	(61)	(62)	(56)
d	0.9848(15')	0.9905($\frac{56}{15}$)	0.9834(11')
α_D	-0° 50'	-6.159°	-5.2°
n_D		1.5007	1.4874
A.V.	1.8	4.247	0.42
E.V.	12.9	33.75	
S.V.		37.997	28.83
Did not congeal		at -5° to -6°C.	at -10°C.
Soluble in	5-6 vols. alcohol 80%	9 vols. alcohol 80%	

EXPERIMENTAL

Distillation Studies of the Fresh Leaves: To arrive at a quantitative estimate of the oil present in the leaf, the apparatus described by Middleton and Cocking (63) was used. In general, the procedure was as follows: 40 to 50 gm. of the fresh leaf was added to about 400 cc. of water, previously brought to a boil in the heating flask. The flask was then connected to the condenser-receiver and was gently rotated until the water was again boiling freely. After two hours heating the flame was removed and the volume of oil

read. In Table 5. the results obtained by these distillation tests are set out.

Table 5.

Date	Source of Leaf	No. of Determinations	Per cent Yield of Oil
Oct. 11, 1938	Gainesville	4	0.21
Feb. 7, 1939	West Florida	2	0.50
Mar. 13, 1939	Gainesville	2	0.19
July 10, 1939	Gainesville	3	0.16

In order to obtain additional oil for experimental work from the leaf collected in Gainesville a steam still was set up in the laboratory, in which the charge was enclosed in a 5 L. pyrex flask. From 1650 gm. of fresh leaf an average yield of 0.11 % was obtained, October 25, 1938. In the case of the West Florida leaf, which was available in larger quantity, an all-metal still connected directly with the steam line was used. A batch of approximately 10 Kg. was distilled and a yield of 0.335 % of oil obtained, including that reclaimed by cohobation of weak distillates. For the purpose of comparison 1200 gm. of this same leaf was distilled in the all-glass still, in which case the yield was 0.325 %, also including cohobated oil. These latter distillations were conducted February 7 and 8, 1939.

Upon examination the oil distilled from leaf collected in October was found to have d_{4}^{20} 0.8788; n_D^{20} 1.4723 and an ester value of 96.41, whereas for the oil obtained in February from West Florida leaf the following constants were determined: d_{4}^{20} 1.0490; $\alpha_D - 0.91^\circ$; n_D^{20} 1.5207; A.V. nil; E.V. 11.31; E.V. after acetylation 85.27. Upon carrying out an acetylation by the ordinary method a value of only 46.6 was obtained.

The sample of West Florida oil was at first almost colorless but gradually acquired a light yellow color. It possessed a characteristic pleasant and refreshing odor, although possibly less so than the oil distilled in October. When tested by the method of sodium fusion no nitrogen or sulfur was found to be present.

Phenols: A 10 cc. sample was shaken in a cassia flask with solution of KOH and allowed to stand in contact with it over night. No absorption was noted.

Aldehydes and Ketones: The oil gave a faintly positive test with Tollen's reagent but was negative with Schiff's reagent and with the test of Rodionow and Korolew. A small sample examined with semicarbazide reagent by the micro-method, and a larger sample treated with sodium bisulfite, both failed to show evidence of the formation of an addition product.

Saponification and Fractionation: A preliminary micro boiling-point determination indicated a high boiling range. 15 cc. of the sample was then heated on a water-bath for 1 hour with 15 cc. of half normal alcoholic potash, after which the greater part of the alcohol was distilled off and the oil

was then washed and dried over anhydrous sodium sulfate and fractionated under 15 mm. pressure with the results shown in Table 6.

Table 6.

Fraction	Volume	B.P.°C.	B.P.°C.	d ₄ ²⁰	n _D ²⁰
		15 mm.	760 mm.		
1	0.5 cc.	62	190		1.4650
2	0.75 cc.	73-74	242	0.9515	1.5068
3	2.5 cc.	121	270	1.1114	1.5385
4	4.3 cc.	126-130	275	1.1331	1.5399
Residue	1.0 cc.				
Total	9.05 cc.				

Fraction No. 1. Possessed the characteristic odor of the original sample. When cooled to a low temperature with dry ice (below -30°C.) the liquid became exceedingly viscous but without crystal formation. The total amount available was used in an unsuccessful attempt to prepare and separate a phenylurethane.

Fraction No. 2. The odor appeared to be somewhat different from that of the above mentioned fraction. When cooled to a low temperature there was a separation of crystals following which the whole set into a solid mass. The melting-point appeared to be approximately 0 to 2°C. No phenylurethane could be obtained.

Fraction No. 3. This fraction was almost devoid of odor and when cooled congealed at -17°C . to such an extent that no liquid could be poured off. The melting-point in this case was approximately 5 to 8°C . 1 cc. of this fraction was then examined for safrol by the method employed successfully by Foote (59) but no piperonylic acid was obtained. When this same procedure was applied to a known sample of safrol, piperonylic acid (m.p. $227-228^{\circ}\text{C}$.) was obtained.

Fraction No. 4. This fraction was likewise almost devoid of odor and the constants (see above) differ but slightly from fraction No. 3. The congealing point in this case was also identical and the melting-point may have been slightly higher ($8-10^{\circ}\text{C}$.). Some liquid was at first separated during the congealing but when again chilled this also solidified and further examination showed it to have constants not appreciably different from the original fraction. 2 cc. was then tested for isosafrol by the method of Wallach and Muller (64) but no isosafrol nitrite could be separated.

DISCUSSION OF RESULTS

From the yields observed as a result of quantitative determinations it is apparent that the proportion of volatile oil present in the leaf of I. floridanum varies at different seasons of the year. In October, again in March, and in July the yield was approximately 0.20 %. Variations from this

figure reported in Table 5. must be regarded as within the limit of error in reading the amount of oil in the measuring arm of the receiver. In February, on the other hand, the yield of oil had apparently risen to 0.50 %. It must be said however, that there are two factors which may have some bearing on this high yield. In the first place this leaf (collected in West Florida) contained a small proportion of twigs, and secondly, distillation was not carried out until several days after collection. The distillation yields also indicated that by the methods of steam distillation, such as might ordinarily be employed in the laboratory, all the oil present in the leaf is not recovered. When 0.21 % was present only 0.11 % was obtained and when 0.50 % was present a maximum of 0.335 % was recovered, even when the weak distillate was cohobated.

More striking than the seasonal variation in yield of oil is the widespread between the physical properties of the oils distilled in October and in February. During this interim the specific gravity underwent a transition from 0.8788 to 1.0490 and the refractive index from 1.4723 to 1.5207. To check the possibility of an environment factor in this regard, as soon as the increase in density was noted, a small sample of leaf was collected from the Gainesville source and distilled (Mar. 13, 1939) in Middleton and Cocking's apparatus. In this case also the oil appeared to be denser than water and sank to the bottom of the measuring arm in the receiver.

Coincident with the above noted change in physical properties there was a decline in the ester value from 96.41 in October to 11.31 in February. The limited sample of the former oil did not permit a further comparison but with the latter an ester value after acetylation of 85.27 was noted (46.6 when determined by the ordinary method). This last observation apparently points to the possibility of a tertiary alcohol and it is to be noted that linalool has been reported (61) as a constituent of the oil of I. religiosum. The observed boiling-point and refractive index, (b. p. 198 - 199 °C. /760 mm., n_D^{20} 1.4668), of the very small fraction No. 1 correspond closely to those reported (6) for linalool but no phenylurethane could be obtained for confirmation.

The specific gravities and refractive indices of fraction 3 and fraction 4 are in good agreement with those of safrol, for which Eykman (65) reported d_4^{20} 1.0960 and n_D^{20} 1.5420. It was also noted that, when solidified, these fractions melted 5 to 10 C. (Eykman gives 8 °C. for safrol). In view of the fact that safrol has been reported (59) (60) (61) as a constituent of the closely related oils I. parviflorum and I. religiosum, it is a likely possibility here. However, the boiling range over which these two fractions were obtained is higher than that usually reported for safrol and furthermore oxidation by alkaline permanganate failed to yield piperonylic acid. The higher density of fraction 4 together with the fact that the oil was boiled with alcoholic KOH prior to fractionating suggest the possible presence of

isosafrol in this fraction but the refractive index does not bear this out. Furthermore when tested for isosafrol by the method Wallach and Muller no nitrite was obtained. However, there is distinct evidence of the presence in these two fractions of some substance of higher boiling-point and higher density than safrol and which refractionation of a larger sample might definitely establish.

Likewise there is some doubt as to the existence of a true fraction in between these high boiling fractions and the fraction of 190 C. The refractive index of this portion of the distillate is intermediate between fraction 1 and fraction 3 and the specific gravity was considerably less than that of fraction 3. However, the behavior under cooling was very suggestive of the latter fraction.

On the basis of the physical evidence presented it would appear that in I. floridanum a high boiling-point, high gravity constituent or constituents, develops in the oil with the approach of the fruiting stage, apparently at the expense of the esters present in the oil earlier in the season.

P A R T I I

PEPPERMINT OILS (1929-1932)

Chapter 1.

REVIEW OF THE LITERATURE

Early History of Peppermint:

Down through the ages "mint" has always occupied a position of prominence in the list of useful plants. It is known to have been grown in Egypt three thousand years ago, since dried peppermint leaves have been found by archaeologists in the tombs of that period and carvings of therapeutic plants on the walls of the temple of Karnak (1500 B. C.) included the peppermint plant. It cannot be determined today whether the peppermint cultures of the ancient Egyptians consisted of Mentha piperita or a related species. The cultivation of mint in Japan cannot be traced authentically to its origin but it is said to have been known in that country over two thousand years ago. A. M. Todd, one of the founders of the peppermint producing industry in this country has compiled a detailed record (66) of the origin of the terminology and references to mint in the writings of the ancients.

Modern interest in peppermint can be traced to 1696 when Ray described the plant in his "Synopsis Stirpium

Britannicum" (2nd. ed.). It appears that his attention was drawn to it by a Dr. Eales who observed it growing in Hertfordshire, England. Ray's original specimen is reported to be still in the British Museum. In 1721 peppermint was admitted to the London Pharmacopoeia as "Mentha piperitis sapore", and by the middle of the 18th century it was being widely cultivated, especially around Mitcham, in Surrey.

The Peppermint Industry in the United States:

It is not unlikely that New England settlers brought the plant to this country. According to Todd (66) the industry in America may be said to have been started in 1816 by Burnett, who collected a quantity of plants on the shore of a stream in Wayne County, New York State, and distilled therefrom about forty pounds of oil. H. Kraemer (67) also credits Burnett with starting the industry but states that he was a peddler who bartered his wares for peppermint when the farmers were short of funds. Soon he began to secure such quantities of the herb that he built several stills in Wayne County and then gave up his peddling business and devoted himself to collecting and distilling the mint. The industry thrived and Wayne County soon became one of the chief peppermint producing centers of the world. As early as 1835, however, the black muck lands of southwestern Michigan and northern Indiana were found to be well-suited for mint culture and at the present time about ninety per

cent of the American production, representing probably at least three-fourths of the world's supply, comes from this section. According to Sievers (68) no oil of peppermint has been produced in New York State for some time. About twenty-five or thirty years ago cultivation of peppermint was started on a seemingly suitable soil type in western Oregon and Washington. This region now furnishes almost all the balance of the American production, with the exception of a small amount produced in Ohio. The commanding market position achieved by American peppermint is undoubtedly due in large part to improvements in methods of distillation leading to greater yields and a high quality of oil. The data given in Table 7 is compiled from recent reports (69), (70) and is included to indicate the extent and present distribution of the peppermint oil industry in the United States.

Table 7
American Peppermint Oil Output

State	Acreage under Cultivation			Production		
	1937	1938	1939	1937	1938	1939
Indiana	11,600	9,000	9,500	319,000	225,000	285,000
Michigan	18,000	16,700	15,900	450,000	551,000	429,000
Ohio	700	400	240	20,000	8,000	8,000
Oregon	1,900	2,150	2,100	66,000	77,000	84,000
Washington	700	820	820	24,000	29,000	39,000
Total	32,900	29,070	28,560	879,000	890,000	845,000

Cultivation and Volatile Oil Content:

Peppermint thrives best in deep soils which are rich in humus and retentive of moisture, but fairly open in texture and well drained. It may also be grown successfully in well-prepared upland soils. From a practical standpoint there is now considerable information available regarding the external conditions which will enable the grower and distiller to produce the best results, both qualitatively and quantitatively, with respect to the volatile oil. One of the most complete of the early investigations in this country was conducted by F. Rabak (71). Rabak studied the effect of cultural and climatic conditions on the yield and quality of peppermint oil in order to obtain data bearing on possible variations

in the composition of the oil under varying conditions of soil and climate and as a result of harvesting at different stages in the plants development. From the results obtained and from the observations of other workers, Rabak concluded that light soils of either sandy or loamy nature are more conducive to the production of esters and menthol in the oil than soils of heavy texture. He also noted that the yield of oil distilled from the fresh plants apparently decreases as the plant matures while the yield from the whole herb in the budding and flowering stages is consistently lower than that from the leaves and tops. He reports that drying the herb before distillation resulted in considerable loss of oil and caused changes favorable to esterification. However, notwithstanding this higher ester content the odor of the oil from the dry plants was judged to be decidedly inferior. The effect of shade upon the peppermint plant was also included in this study and the conclusion was that it caused a decrease in esterification and formation of free menthol. It was suggested that this was due possibly to lessened activity of the elimination of water by the plant. The action of frost, on the other hand, was found to be in the direction of increased esterification and menthol formation.

More recently, M. B. Sardanovsky (72) has published the results of a research into the changes in the composition

of oil of peppermint with the different stages of the plant's growth. This work was carried out at the Experiment Station at Lubny (U. S. S. R.). In this case it was noted that the volatile oil content in the peppermint stalks falls steadily during the second year of cultivation and disappears entirely before the flowers have fully matured. In the leaves the oil accumulates with the increasing age of the plant and reaches its maximum in the interval between the formation of the inflorescence and the opening of the flower.

Much of the recent literature on peppermint culture relates to various studies to determine the influence of different fertilizers and soil cultures on the growth of the plant and composition of the volatile oil. Earlier workers in this field were Charabot and his co-workers and Gustav Mossler (7). More recently Boshart (73), Maoku (74), Schlemmer and Springer (75), and Bauer (76), have published their findings. This aspect of peppermint cultivation is being reviewed in a study currently being conducted by Geo. M. Hooking in the Department of Pharmacognosy, University of Florida.

Further reference to the relation of maturity of the plant to free and combined menthol in the oil will be made in connection with the discussion of biogenesis of Oil of Peppermint.

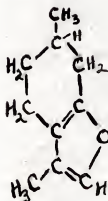
The Constituents of Oil of Peppermint:

The classical work of Power and Kleber (77) was responsible for the identification of no less than seventeen different constituents in American Oil of Peppermint. Since that time workers in the laboratory of Edward Kremers, Madison, Wisconsin, have added several others. Thus in Gildemeister and Hoffmann (78) twenty-two constituents are now listed, as follows:

1. Acetaldehyde
2. Isovalerianic aldehyde
3. Free acetic acid
4. α -Pinene (inactive)
5. Free isovalerianic acid
6. Phellandrene
7. Cineol
8. 1-Limonene
9. 1-Menthone
10. 1-Menthol
11. Menthyl acetate
12. Menthyl isovalerianate
13. Menthyl ester of an acid $C_8H_{12}O_2$.
14. A Lactone, $C_{10}H_{16}O_2$.
15. Cadinene
16. Amyl alcohol
17. Dimethyl sulfide

18. Pulegone (and its hydrolysis product
1,3-methylcyclohexanone)
19. Δ^1 -Menthenone-3
20. An isomeric menthenone
21. Terpinene
22. d-Menthene

Although this list was compiled in 1931, it does not include a new constituent reported in 1929 by Carles (79) to have been isolated from the oil obtained by steam distilling fresh herb of flowering peppermint. From the fraction distilling between 70-75°/10 mm., after removal of menthol, there was obtained an oxygenated oil possessing an odor of peppermint flowers. It had the following constants: n_{D}^{20} 0.965; n_D^{20} 1.4807; $\alpha_D + 81^\circ$; b. p. 95°/20 mm. (196°/760 mm.). This constituent was later identified by Wienhaus and Dewein (80). They prepared it in a pure state and found its empirical formula to be $C_{10}H_{14}O$. From an



Menthofurane

examination of oxidation and reduction products its constitution was established as 3,6-dimethylcoumarone tetrahydride (4:5:6:7). It was named "Menthofurane".

In 1937 Rao and co-workers (81) reported that from an oil distilled in India from Mentha

piperita L. optically inactive menthol (m. p. 33) was isolated. This they considered to indicate the presence of isomeric menthols.

In 1940, Janistyn (82) reported that esters of hexenol occur in peppermint oil.

The Occurrence of Pulegone in Oil of Peppermint:

In reporting to the meeting of the Bernese Botanical Society, October 23, 1922, Tschirch (83) observed that for several years past samples of Mentha piperita which had been sent to him no longer smelled of menthol, but reminded of pennyroyal (pulegone) or of spearmint (carvone). Tschirch found an explanation of this fact in the dehybridization of the hybrid forms. As the plants of Mentha piperita have been unable to regenerate themselves within the past two centuries, the original hybrid, which contained menthol, has reverted to the ancestral forms: Mentha aquatica, containing pulegone; and Mentha viridis, containing carvone.

1,3-methylcyclohexanone and acetone are the products of the hydrolysis of pulegone. The occurrence of the first in the 'vorlauf' of the cohobated oil of American peppermint and the second in the cohobated aqueous distillate led R. E. Kremers (84) to assume that pulegone was their forerunner in the elaboration of the oil by the plant. A menthone fraction, partially freed from menthol by freezing, of the oil which had given these results was therefore examined for

pulegone and its presence definitely established.

A number of references have been noted to apparently abnormal peppermint oils. Although pulegone in none of these instances is reported as a constituent, the constants cited are suggestive of its presence.

1. In 1930 two samples of Cyprian oil of peppermint examined in the Imperial Institute in London were found (85) to have the following constants: d_{15}° 0.961, 0.937; n_D^{20} 1.482, 1.482; $\alpha_D + 14.5^{\circ} + 22^{\circ}$; E. V. after acetylation 132.5, 93.0; total menthol content 41%, 27.9 %.

2. An oil obtained from plants grown in Hungary showed (86) the following constants: d_{15}° 0.9167; n_D^{20} 1.4619; $\alpha_D + 34.14^{\circ}$; A. V. 0.85; E. V. 80.72; combined menthol 22.19 %; total menthol 75.54 %; menthone 1.47 %.

3. A peppermint oil from Tanganyika Territory, obtained from Mitcham plants transplanted there, showed (87) quite different behavior from the commercial oils. This oil was almost colorless and had the following constants: d_{15}° 0.9346; n_D^{20} 1.4674; $\alpha_D^{20} + 10.36^{\circ}$; A. V. 0.7; E. V. 50.6; E. V. after acetylation 149.5 (corresp. to 46.9 % total menthol).

4. The herb of Indian peppermint, Mentha piperita, L., grown near Bangalore is reported (81) to have yielded a yellowish brown volatile oil with the following constants: d_{15}° 0.9285, 0.9354; n_D^{20} 1.4754, 1.4758; $\alpha_D + 24.0^{\circ} + 32.6^{\circ}$; A. V. 0.5, 1.2; E. V. 16.7, 21.4; free alcohol (as menthol)

19.1 %, 20.5 %. It is pointed out in this case that the dextro-rotation of the oil is noteworthy and the density and high refractive index are likewise unusual.

The Biogenesis of Oil of Peppermint:

Mentha piperita L. is now considered to be a triple hybrid between M. sylvestris L., M. rotundifolia L., and M. aquatica L. This modern concept has arisen as a result of the cytological studies of Schürhoff (88) and other workers who have found that M. viridis L. is itself a hybrid of M. sylvestris and M. rotundifolia. Schürhoff observed that M. piperita plants from different localities differed from each other appreciably. It was possible, indeed, to classify such plants into those which approached more closely to M. viridis and those which showed greater resemblance to M. aquatica. Thus a continuous series of the various hybrids was arranged all of which were intermediates of M. viridis and M. aquatica. This, according to Schürhoff, has considerable significance insofar as the volatile oil is concerned because it has been observed that when the plant externally resembles M. aquatica its odor is more pleasant while the nearer it approaches M. viridis the more unpleasant and the less refreshing the odor becomes.

Meyer (89) corroborates Schürhoff's conclusion that M. aquatica, both in ordinary peppermint and in Japanese

peppermint, seems to give the impulse to the formation of menthol. Since a third hybrid of M. aquatica, M. verticillata, forms no menthol, he contends that from this it may be concluded that the predisposition to menthol formation is not inherited through the chromosomes, but through the genes present in the cell plasma. Thus predisposition to menthol formation is only met with in such hybrids where M. aquatica played the role of mother plant in the origin.

Mentha piperita and other mints have frequently received the attention of those whose desire it was to study the biogenesis of cyclic compounds, especially those belonging to the terpene group. As R. E. Kremers (90) states, the hybrid nature of the mints provides excellent opportunities for the comparison of morphological with chemical hereditary characteristics.

Close structural relationships between the individual components of a particular volatile oil have often been pointed out. It has also been realized that parallels may be drawn in many instances between groups of substances occurring in two or more oils from related species. Thus the idea of a common mechanism of formation arises, by which related substances are derived in the plant from common chemical ancestors. In this connection Hall (91) states that: "Any phytochemical generalizations limited to data

obtained from a species, a genus, or even a family are questionable. The coexistence of compounds in a few instances out of many, for example, may indicate nothing as to their biogenetic relationship; their coexistence in a large percentage of their occurrences may indicate, not so much their mutual transformation one into the other, as their derivation from a common source."

Several theories of biogenesis have been proposed which may be mentioned very briefly here. The possibility that terpenes and their oxygenated derivatives are built up in the plant by processes involving aldol and true condensation of acetaldehyde and acetone is one of the oldest points of view. The suggestion of Smedley (92), in 1911, that citral is formed in the plant from two molecules of acetone and two of acetaldehyde has been elaborated by R. F. Kremers (90) in applying this theory to the mints. The theoretical scheme which Kremers proposed in 1922 was intended to account for the various chemical relatives found in the three botanically closely related species of mentha, M. aquatica, M. viridis, and M. piperita. This scheme, outlined in Figure 1, has been accepted and published by several other authors.

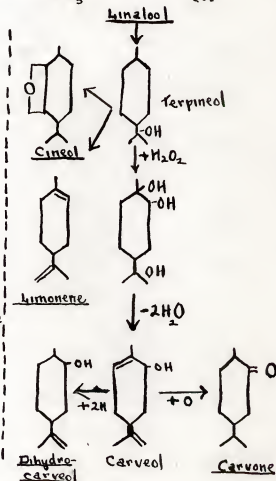
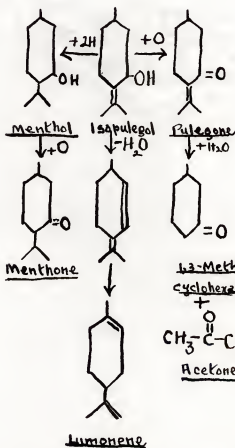
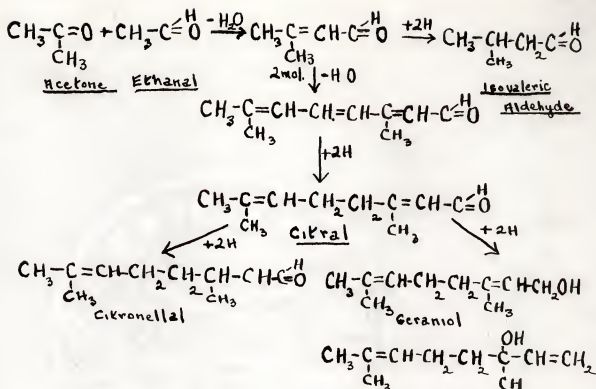


Figure 1

— denotes compounds known to occur in the volatile oils.

It will be observed that the immediate common ancestor of the two series of compounds in Figure 1 is citral. The whole scheme hinges on the conception that the biochemical conditions which in spearmint bring about the reduction of the aldehyde group are modified in peppermint so as to cause the reduction of an ethylenic linkage. This differential reduction of citral may be accomplished in the laboratory, as pointed out by Read (93), by using (1) sodium amalgam and acetic acid, and (2) hydrogen in the presence of colloidal palladium.

In 1930, Read (93) observed that some of the most arresting biogenetic relationships in the menthone series center around piperitone. Piperitone has been found to be invariably associated with geranyl acetate in Eucalyptus oils. On the basis of theoretical evidence Read contends that geraniol possesses strong claims to be regarded as the immediate precursor of piperitol, piperitone, Δ^4 -carene and other substances which occur in this association in volatile oils. He states: "the implied aptitude of geraniol to function in nature as a precursor of so many other substances may be attributed largely to the unusual conformation of its molecule, which possesses a structure (A), $\text{.CMe:CH.CH}_2\text{OH}$, consisting of a primary alcohol group activated by an $\alpha\beta$ -ethylenic linkage and situated in spatial proximity to a second active grouping (B), .CH:CMe ,

containing another double bond".

Hall (91) contends that the derivation of all terpenes from geraniol requires too much shifting of double bonds and rearrangement. Assuming phytochemical processes to be more direct, he presents the idea that it is more logical to attempt to refer to all known terpene configurations to underlying fully hydroxylated compounds.

Isoprene has also been suggested as the building unit of primary importance in the plant synthesis of terpene hydrocarbons. Two recent authors, Malowan (94) and Dodge (95) discuss its possible significance. Malowan shows diagrammatically the formation of geraniol, linalool, and terpineol from isoprene. Dodge gives a schematic presentation of the building up of the various types of terpenes from isoprene.

Of more direct concern to this present investigation than general hypotheses of biogenesis is the problem of menthol-menthone relations in Mentha piperita. A number of investigators have attempted to follow the menthol-menthone ratio through the various stages of the plant's development and their results have been used to formulate certain theories. In 1900 Charabot (96) and his co-workers began a series of such experiments from which several conclusions were drawn: (1) At the beginning of the vegetation of the mint the oil is rich in menthol, a small part

of which is in the combined state; (2) menthone exists only in minimal amounts at this period; (3) the menthone increases with the vegetation process at the expense of the menthol, the total menthol-menthone content remaining constant; (4) the oxidation takes place chiefly in the inflorescence; (5) the quantity of esters and terpenes increases, probably due to a diastase of reversible action, which functions as a dehydrating body.

Some years after this earlier work of Charabot Kleber (97) examined the oils from peppermint plants of different ages and found that it was the oil from the younger plants that contained the highest proportion of menthone. This implies a reduction and not an oxidation process in the plant.

This observation of Kleber seems to be borne out by the recent work of Rutevski and Travin (98), as indicated in Table 8.

Table 8
Menthol-Menthone Relationship at Different
Vegetative Stages *

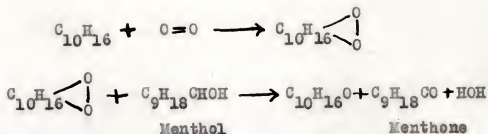
Date Collected	Vegetative Stage	A. V.	E. V.	E.V. (after Combined Free acetyl.)	Per cent		Per cent
					Menthol	Free Menthone	
July 17-19	long before flowering	0.49	29.46	158.64	8.21	39.67	13.04
Aug. 8	no flower buds	0.81	26.05	170.51	7.25	44.85	
Aug. 14	with flower buds	0.64	28.61	176.39	7.97	46.07	6.38
Aug. 31-Sept. 3	start of bloom	0.75	40.00	195.73	11.16	48.82	7.22
Sept. 17-18	full bloom	0.47	48.11	195.97	13.04	46.33	1.43
Oct. 3	past bloom	0.64	56.28	201.02	15.68	45.00	2.46

* Rutevski and Travin (98) from plants raised at the Experiment Station Ogino of the State Chem.-Pharm. Research Institute at Moscow, U.S.S.R.

In 1912, Brooks (99) established the presence of an oxidizing enzyme in peppermint, located chiefly in the inflorescences. This was confirmed by Gordon (100) who quantitatively determined catalase and peroxidase values in the growing peppermint plant. He found that both of these reached a maximum at the flowering period. Just past the flowering period the enzymes took a decided drop. It was further demonstrated that the florets alone showed little oxidizing capacity in terms of catalase and peroxidase values. This Gordon points out, is contrary to Charabot's statement that the seat of oxidation of menthol is in the

inflorescence, if a direct oxidation is involved. Consequently the rather satisfactory explanation is advanced that Charabot's observations, which also are in conflict with those of Kleber (and now with those of Rutovski and Travin) may be brought into harmony by assuming that oxidation, if the oxidizing enzymes are involved, takes place in the leaf, and that the oxidized product, menthone, is transported to the flower, thus disturbing the equilibrium.

The data of Charabot with respect to increased content of unsaturated hydrocarbons in the inflorescences is also of significance. Gordon (100) noted that Charabot also expressed the opinion that at that vegetative stage increased oxygen absorption is taking place. This, he contends, should be considered in the light of recently advanced auto-oxidation theories wherein unsaturated bodies act as carriers of oxygen through intermediate peroxide formation. Postulating a reaction whereby a system made up of peroxide, peroxidase, and free oxygen, apparently simulating the condition at the flowering period, there is stated to be the possibility that such a system might oxidize a secondary alcohol to a ketone. Thus, Gordon assumes that the peroxide $\left(\begin{smallmatrix} \text{O} \\ \text{A} \text{---} \text{O} \end{smallmatrix} \right)$, acting under the influence of a peroxidase, might oxidize menthol to menthone in the following sense:



However the negative results of about twenty experiments indicated that the oxidizing enzymes isolated from Mentha piperita, alone or in the presence of so-called terpene peroxides, apparently simulating conditions in the plant, do not possess the power of oxidizing menthol to menthone. Thus there seemed to Gordon to be no relation between the presence of considerable amounts of oxidizing enzymes in the plant and a direct oxidation of menthol.

On the other hand there is a certain amount of laboratory evidence to show that menthol may be produced from menthone by a process that might possibly be duplicated in the plant. Pickard and Littlebury (101), in 1912, suggested that l-menthone is first formed in the plant but is afterwards reduced to a mixture of l-menthol and d-neomenthol, the l-menthol being formed in very much larger proportion. Gordon (102) was able to bring about the formation of a small amount of menthol by a modified Cannizzaro reaction. He records a crossed dismutation between benzaldehyde and menthone by means of aluminium ethylate. In a non-aqueous medium the benzoic acid ester of menthol was obtained, which on hydrolysis yielded menthol and benzoic acid. Since

acetaldehyde and other aldehydes have been found to accompany compounds of ketonic nature in the plant kingdom, it is suggested that this form of reduction may be of special significance in biogenesis.

Chapter 2.

THE DETERMINATION OF PHYSICAL AND CHEMICAL CONSTANTS

Table 9.

Physical and Chemical Constants Peppermint Oils (1929-1932)

Sample	d_{20}^{20}	n_{20}^{20}	α_{20}^{20}	A.V.	E.V.* (Calc. as Menthyl. Acetate)	E.V. after acetyl* (Calc. as free Menthol**)
# 2.						
Found	0.9275	1.4838	+13.65°	2.33	13.10(4.63%)	36.49(8.55%)
Hiner	0.9213	1.480	+16.89°	0.79	11.07(3.07%)	41.7(8.55%)

# 3.						
Found	0.9251	1.4865	+15.81°	2.31	13.20(4.67%)	19.70(4.89%)
Hiner	0.9266	1.4815	+16.20°	0.72	6.89(1.92%)	32.31(9.22%)

# 4.						
Found	0.9311	1.4860	+16.96°	2.10	10.91(3.87%)	43.95(9.49%)
Hiner	0.9272	1.4801	+16.35°	0.70	6.40(1.79%)	52.58(15.15%)

# 5.						
Found	0.9298	1.4871	+15.53°	1.15	10.35(3.66%)	30.23(5.68%)

# 6.						
Found	0.9192	1.4670	+ 0.24°	2.04	30.94(10.94%)	138.00(33.44%)

# 7.						
Found	0.9351	1.4880	+17.41°	2.28	11.68(4.13%)	35.58(6.82%)
Hiner	0.9263	1.4838	+16.40°	0.77	8.02(2.23%)	25.13(7.23%)

* U. S. P. XI Method

** Calculated according to (6) i.e. % free menthol = menthol in original oil $\times (1 - \frac{E}{E_1})$

The particulars with respect to the origin of the several peppermint oils investigated have been given in Table 1. (see p. 6). The results of the determination of their physical and chemical constants are set out in Table 9. The constants reported by Hiner* for some of these oils are also indicated.

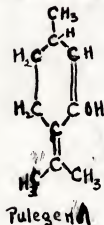
The results for the physical constants agree reasonably well with those expressed by Hiner for the same sample. There is a marked increase in acid value, as is to be expected considering the time interval. The ester values found are consistently higher than those recorded by Hiner and the ester values after acetylation lower.

In 1929 Werner (1), working with the oil of Pycnanthemum muticum (*Koellia mutica* Michx.), an oil found to be rich in pulegone, observed that the presence of this constituent led to abnormally high ester and free alcohol values. Upon investigation he found that pulegone itself gave an apparent ester value of 10.95 (3.87% alcohol $C_{10}H_{20}O$, calculated as acetate) and an ester value after acetylation of 61.29 (17.90% of free alcohol, calculated as $C_{10}H_{20}O$). This anomaly was also noted by Hiner but its significance seems to have escaped others. For instance Kremers (103) working with oil of Mentha canadensis L. reports a pulegone content of 90 %, E.V. 11.2, E.V. after acetylation 33.6 (6.5 % free alcohol). Jenison and Kremers (104) with oil of Japanese peppermint report a pulegone content of 86 %, ester content 6.6 %, and free alcohol 12.1 %. In each case, however, it is stated that

* M. S. Thesis, University of Florida, 1931.

no menthol could be detected in 1 liter of oil examined. It will be noted that ester and free alcohol contents reported are lower than the apparent values Werner obtained with pulegone alone.

It has long been known to be theoretically possible for ketones of the terpene series to exist in the enol form. In 1927 Gordon (105) discussed the existence of menthone in the enol form. Simonsen (106) states that pulegenol, the enolic



form of pulegone, is comparatively stable and can be distilled without change. The following constants are recorded: b.p. $85^{\circ}\text{C.}/6\text{ mm.}$, $d_4^{20} 0.916$, $n_D^{20} 1.48312$, $[\alpha]_D^{20} +24.6^{\circ}$. Simonsen also points out that by distillation in steam or by action of alkalis pulegenol is isomerized to the ketone.

Schmidt (107) has studied the preparation of various enol acetates and he reports enolization of cyclic ketones to proceed with some difficulty. However, he gives a method by which pulegone enol acetate was prepared.

To check Werner's observation regarding apparent ester values for pulegone, a sample of this ketone, regenerated from sulfite addition product of an original pulegone fraction from oil # 2 was assayed by the U. S. P. XI method with the following results:

E.V. 7.02, 7.39 (Av. 7.21).

E.V. after acetylation . . 34.80, 33.14, 34.14
33.19 (Av. 33.82).

Thus it has been confirmed that pulegone itself is capable of yielding ester values both before and after acetylation. It, therefore, seems likely that a peppermint oil containing pulegone in addition to menthol might give an abnormally high result and that an oil containing much pulegone and little or no menthol might appear to contain an appreciable amount of menthol when assayed by standard methods.

The formation of an enol acetate during acetylation would appear to explain the increased ester value in that instance. The apparent ester value is explainable on the basis that in the presence of pulegone undue resinification or polymerization is induced by prolonged heating with potassium hydroxide, some of the base being used up.

Brignall (16) has recently devised a rapid method for determining free menthol which is claimed to avoid some of the errors of the standard method. In reviewing some of the disadvantages of the official method he points out that it is known to indicate as alcohols such non-alcohol constituents as aldehydes, amines, phenols, esters, and some of the more unstable terpenes which are acetylated along with the alcohols. He also refers to a fact observed here, in the case of the abnormal oils, that badly oxidized or poor quality samples are difficult to analyze consistently owing to discoloration during saponification which interferes with observation of the endpoint.

From data presented in several tables, Brignall shows

that by his method for assaying oil of peppermint results are obtained which average about three per cent lower than those found with the official method. This divergence is traced, in the case of one particular sample, to small quantities of aldehydes contained in the lower boiling fractions, and to polymerization during saponification of the higher boiling fractions, with a subsequent utilization of alkali.

The application of Brignall's rapid method to the abnormal oils under investigation has been studied. By this method all of the oils were re-analyzed for free alcohols and the results are given in Table 10. The method employed was as follows: 1 gm. of the sample, accurately weighed in a tared acetylation flask, was treated with 5 cc. (accurately measured by means of a Koch microburette) of a freshly prepared acetylating mixture consisting of four parts by volume of n-butyl ether and one part acetic anhydride. A blank was prepared in an identical manner omitting the oil. The flasks were then connected to air condensers and the contents boiled gently for 1 hour on a sand-bath. Without removing the flasks from the sand-bath, 20 cc. of hot distilled water was added through the condensers and the contents boiled vigorously for an additional 30 minutes to convert the excess anhydride into acetic acid. After removing the flasks from the sand-bath and allowing the contents to cool to room temperature, 20 cc. of distilled water was added as before. The flasks were then removed from the condensers and the ground-glass

connections were rinsed in such a manner as to allow the rinsings to flow into the flasks. After adding 8 to 10 drops solution of phenolphthalein the excess acid was neutralized with 0.5 N alcoholic KOH. The blank was titrated to the full red color of the indicator and the oil sample matched with the blank. The calculation was made as follows:

$$\% \text{ free alcohol} = \frac{\frac{\text{Molecular wgt. of alcohol}}{20} \times A}{\text{Wgt. of sample}}$$

Where A = difference in the amount of 0.5 N KOH used in the blank and in the sample (corrected for the amount of base required to neutralize the free acid in an equivalent amount of the oil).

The results indicated in Table 10. tend to support Brignall's contention that lower values are obtained by this modified method, presumably because certain errors of the official method have been avoided. The results with oil # 6 are quite consistent. This is significant because this sample did show fairly good menthol content and contained, as will be shown later (see Table 12.), very little pulegone. With the Washington oils the results are not as consistent but show a lower trend than the average U. S. P. values. Difficulty was experienced in duplicating results in the case of oil # 2. This was lessened by re-drying the oil over anhydrous sodium sulfate.

Table 10.

Comparison of Brignall Method with U. S. P. XI Method

Sample	Percentage Brignall Method	Free Average by U. S. P. Method(see Table 9.)	Menthol Reported by Hiner
# 2	11.53 22.17 8.95* 8.01* 10.08*	8.55	8.55
# 3	2.59 2.60 2.20	4.89	9.22
# 4	4.75 6.44	9.49	15.15
# 5	5.96 7.70	5.68	
# 6	27.14 26.06 24.66 26.44	33.44	
# 7	2.93 2.23	6.82	7.23

* Determined after re-drying sample.

In order to determine the extent of the error due to pulegone when the Brignall method is used two experiments were carried out.

(1) To 1.0315 gm. of oil #6, 0.7039 gm. of regenerated pulegone (from sample # 2) was added and the determination of free menthol carried out by the Brignall method. Result - 26.54 %. (Average from Table 10., 26.08 %).

(2) A mixture was prepared containing a known amount of menthol (approximately equivalent to the content in oil # 6) and an equal amount of the regenerated pulegone. The mixture used contained menthol 30 parts, pulegone 30 parts, menthone 20 parts, d-limonene 20 parts (all by weight). The results of the Brignall assays with this mixture are shown in Table 11. with results obtained using 30 % w/w menthol in d-limonene as a control.

Table 11.

Brignall's Assay Applied to Known Menthol Mixtures

Mixture	Per cent <u>Present</u>	Per cent <u>Found</u>
Menthol (in d-limonene)	30	30.74
	30	29.06

Menthol - Pulegone - Menthone (in d-limonene)	30	27.28
	30	29.80

It is a striking fact that in no instance where pulegone was added was a result obtained that was significantly higher than the control. Thus it would appear that by this modified method of acetylating the menthol there is less likelihood of enol acetates being formed than when the U. S. P. XI method is used. It will be noted that menthone was also included in the last mixture as a factor which might contribute to an abnormal result.

Chapter 3

FRACTIONAL DISTILLATION

Each of the samples was assayed for ketone by the neutral sulfite method (6). The results are given in Table 12.

Table 12
Determination of Ketone Content

Sample *	Volume used	Volume unabsorbed	Per Cent
2	10 cc.	1.3 cc.	87.0
3	10 "	1.2 "	88.0
4	10 "	1.2 "	88.0
5	10 "	1.4 "	86.0
6	10 "	9.7 "	3.0
7	10 "	1.1 "	89.0

* See Table 1.

Two different general procedures were followed with respect to the examination of the main bulk of each of the samples:

Procedure No. 1. Fractional distillation, as with samples 2, 3 and 6.

Procedure No. 2. The oil was first treated with sodium bisulfite solution until addition with ketone was

complete and the relatively small amount of unabsorbed oil, after washing and drying, was then fractionated. This procedure was followed with samples 4 and 7.

Fractionating Columns:

To effect a more complete separation of the components of a volatile oil many types of fractionating columns are now available. Theoretically, the longer the column the more effective the separation will be but against this is the fact that the longer column will require more heat to drive the vapor through it and thus increase the possibility of decomposition. Hence in practice a balance has to be struck between these two factors.

Four different fractionating set-ups were employed during this study. The most satisfactory and certainly the most efficient of these was the recently constructed spiral screen packed column in the School of Pharmacy research laboratory, University of Florida. A brief description of each apparatus is given below.

Set-up No. 1. This included an 18 inch (internal diameter 7/16 inch) electrically heated column. The column proper was packed with small Berl saddles and was sealed to a total condensation partial take-off distilling head. It was designed especially for use in the Naval Stores Laboratory and was arranged so as to permit intermittent take-off when distilling under vacuum. For the co-operation received in connection with the use of this column the author is

indebted to Mr. W. D. Stalkup of the Naval Stores Laboratory.

Set-up No. 2. This included a 24/40 standard taper column which had an over-all length of 18 inches (internal diameter 4/5 inch). It was packed to a depth of 14 inches with Penn State rings. Like the column in set-up No. 1 it was wound to provide for electrical heating. It was used in connection with a return-reflux distillation head and an intermittent take-off receiver, as shown in Figure 2. A Cenco automatic vacuum control apparatus was used.

Set-up No. 3. The column in this case was a short 6 inch water-jacketed Vigreux 24/40 standard taper column. Otherwise the set-up was the same as in No. 2 (See Figure 3). This column was used in conjunction with a water-jacketed rheostat and was found to be of advantage for re-fractionating where it was desirable to reduce the column hold-up to a minimum.

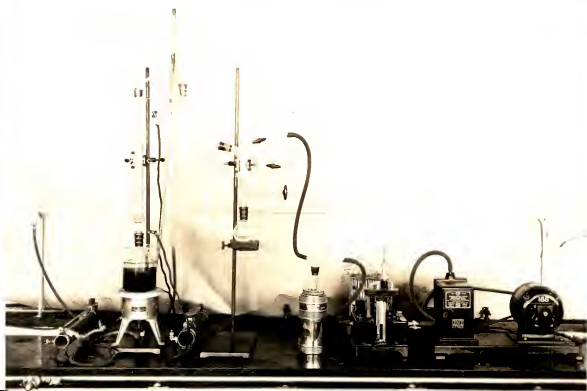


Figure 2

Fractional Distillation Set-up No. 2 (including a column (internal diameter $4/5$ inch) packed to a depth of 14 inches with Penn State rings).

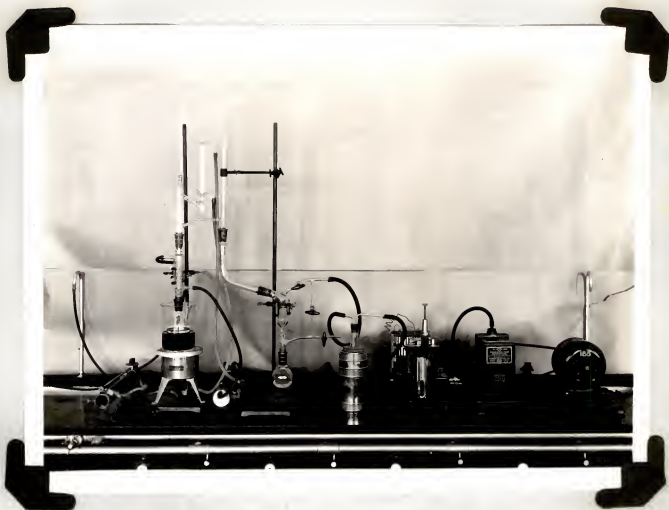


Figure 3

Fractional Distillation Set-up No. 3 (including
a 6 inch water-jacketed Vigreux column).

Set-up No. 4. This has been described in detail by Donate Torres* and is adapted from the original specifications of Lecky and Ewell (108). The automatic pressure control used is described by Hershberg and Huntress (109). For convenience a brief description of the column is repeated here:

The column had an over-all length of $4\frac{1}{2}$ feet (internal diameter $27/64$ inch). The washers had an outside diameter of $7/16$ inch and a center diameter of $3/32$ inch, through which a nickel rod was inserted to provide support and to prevent the vapors rising directly through the center of the packing. The length of the packing was 4 feet and the spacing of the turns was 7 per inch. A heating jacket of common type, controlled by a Standard Electrical Products Co. variable transformer (type SA5, 115 volts, 5 amperes), and two insulating jackets were used. Accurate vacuum readings were made by using a Zimmerli vacuum guage, Precision Model J-1916.

The efficiency of this column was measured by Donate Torres* who reported about 20 plates, using a mixture of carbon tetrachloride and benzene as described by Morton.**

* Donate Torres, D., M. S. Thesis, University of Florida, 1941.

** Morton, A. A., "Laboratory Technique in Organic Chemistry," 1st. Edition, (1938), p. 86.

The Removal of Ketone Through the Bisulfite-Addition

Reaction: Preliminary assays had indicated the presence of a large proportion of ketone capable of combining with sodium bisulfite in all but one of these oils (see Table 12). When it was desired to remove this ketone from the main bulk of the sample, the following process was found to be satisfactory: 500 cc. of the oil was placed in a 5 L. round-bottomed flask with 2 L. of 30 % sodium bisulfite solution, in which the acidity had been reduced by addition of 20 % solution of NaOH. The mixture was heated under reflux on a water-bath for several days with frequent agitation. The greater part of the bisulfite layer was then syphoned off and an additional 200 cc. of the sodium bisulfite solution was added and heating and shaking continued. When the reaction appeared to be complete the unabsorbed oil was removed in a separatory funnel and the bisulfite solutions set aside to crystallize. The crystalline addition product was separated by suction filtration and both the crystals and the mother liquor were washed several times with ether. The ketone was later regenerated.

EXPERIMENTAL

Oil # 2.

With this oil procedure No. 1 was followed. Slightly more than 500 cc. of the sample was shaken out with 5 % solution of sodium carbonate to remove free acids and then

washed with water and dried. Exactly 500 cc. was taken for fractionation. The results of this fractionation are set out in Table 13.

Table 13
Fractionation of Sample # 2 (at 20 mm.)
(Set-up No. 1)

Fraction	Volume	Per cent of whole.	B.P.C. /20mm.	d_{25}^{25}	n_D^{20}	α_D^{25}
1	4 cc.	0.8	below 33	0.9831*		
2	17 "	3.4	33 - 53	0.8481	1.4698	-37.2°
3	18 "	3.6	53 - 76	0.9081	1.4780	+8.7°
4 (a)	270 "	54.0	76 - 83	0.9313	1.4841	+21.0°
4 (b)	83 "	16.6	82 - 83	0.9493	1.4844	+21.0°
Residue	77 "	15.4			1.4978	
Total	469 cc.					

* Received in the cold trap where it is believed to have been contaminated.

Table 14

Refractionation of Fractions in Table 13

(Set-up No. 3 - 5 mm. Pressure)

Fraction	Vol. cc.	B.P.C. 5 mm.	B.P.C. 760 mm.*	d_{25}^{25}	n_D^{20}	α_D^{25}	Per cent Ketone Content**
<u>Fraction 2</u>							
I	4	48-50	155.4	0.8548	1.4659	-16.0°	
II	5	55-58	169	0.8413	1.4712	-48.8°	

<u>Fraction 3</u>							
I	1	60	170.4		1.4774		
II	5	76-80	214.2	0.9227	1.4792	+18.8°	
III	2	84	211.4	0.9636	1.4849		

<u>Fraction 4 (a)</u>							
I	12	78-79	221	0.9291	1.4816	+23.2°	88
II	40	81	221	0.9287	1.4832	+22.3°	46
III	164	83	221	0.9311	1.4850	+21.0°	94

<u>Fraction 4 (b)</u>							
I	67	80	221	0.9384	1.4849	+21.0°	97
II	25	81.5	222	0.9388	1.4860	+16.0°	93

(Continued)

Table 14
(Continued)

Fraction	Vol. cc.	B.P.C. /5 mm.	B.P.C. /760 mm.*	d_{25}^{25}	n_D^{20}	α_D^{15}	Per cent Ketone Content**
Residue (Original)							
I	11	82		0.9347	1.4843	+18.2°	94
II	32	87-88		0.9372	1.4860	+14.0°	88
III	3.5	94			1.4888	+6.6°	
IV	3.0	101			1.4962	-8.0°	59
Residue							

* Corrected to 760 mm. ** Neutral sulfite method.

Note: The residue from each fraction was included in with the succeeding fraction.

Oil # 3.

The free acids were removed, as above, and about 380 cc. of dried oil was taken for fractionation. The results of this fractionation are set out in Table 15.

Table 15

Fractionation of Sample # 3

(Set-up No. 2 - 5 mm. pressure)

Fraction	Vol. cc.	Per cent of whole	B.P.°C. /5 mm.	$d_{\frac{25}{25}^{\circ}}$	$n_{\frac{25}{25}^{\circ}}$	$\alpha_{\frac{25}{25}^{\circ}}$
1	3.4	0.90	below 30"	0.8581	1.4680	- 26.8°
2	16.5	4.34	38-44	0.8407	1.4710	- 43.7°
3	322.0	84.74	75-85	0.9332	1.4859	+ 23.3°
4	6.8	1.79	85-110	0.9382	1.4976	- 9.6°
Residue	20.0	Approximately				
Total	368.7					

* Received in the cold trap.

Table 16
 Refractionation of Fractions in Table 15
 (5 mm. pressure)

Fraction	Vol. cc.	B.P.C. /5 mm.	B.P.C. /760 mm.	d_{25}^{25}	n_D^{20}	α_D^{25}	Per cent Ketone Content
<u>Fraction 2</u> (Set-up No. 3)							
I	2.5	38	157	0.8556	1.4690	-16.8°	
II	7.5	44	168-170	0.8402	1.4706	-55.2°	

<u>Fraction 3</u> (Set-up No. 2)							
I	310.0	83-85	220-223	0.9332	1.4859	+23.3°	98.5

<u>Fraction 4</u> (Set-up No. 3)							
I	13.0	91		0.9380	1.4892	+12.4°	86.0
II	5.0	91-114		0.9451	1.4989	-4.0°	38.3
III	3.6	114-138		0.9649	1.5025	-2.0°	
Residue							

Note: The residue from each fraction was included in with the succeeding fraction.

Oil Unabsorbed by Bisulfite (Samples # 3, 4, and 7).

Procedure No. 2 was followed. The physical and chemical constants were determined for the washed and dried residual oils. The results are given in Table 17.

Table 17

Physical and Chemical Constants of Unabsorbed Oils

Source	Amount Recovered	d_{25}^{25}	n_{20}^{20}	α_{25}^{25}	Esters (calc. as Menthyl Acetate)	Free Menthol*
Oil # 4 (500 cc.)	57 cc.	0.9593	1.4881		26.56%	24.87%
Oil # 7 (500 cc.)	23 cc.	0.9516	1.4965	-13.36°	16.10%	12.76%
Oil # 3 (200 cc.)	15 cc.					

* Brignall Method.

Table 18

 Fractionation of Mixture Unabsorbed Oils *
 (Set-up No. 4 - 20 mm. pressure)

Fraction	Vol. cc.	Per cent of whole	B.P.C. / 20mm.	B.P.C. / 760mm.	d_{25}^{25}	n_{20}^{20}	α_{25}^{25}
1	4	4.9	58	168.5	0.8467	1.4679	-47.04°
2	4	4.9	63	171	0.8399	1.4675	-68.84°
3	6	7.3	81	201	0.8893	1.4559	+10.4°
4	4	4.9	88-89	208-210	0.9080	1.4622	+4.32°
5	4	4.9		209-211	0.9267	1.4750	+2.12°
Residue							

* 82 cc. of the mixture in Table 17 used.

Oil # 6.

230 cc. of the oil was taken for fractionation and procedure No. 1 was followed. The results of the fractionation are set out in Table 19.

Table 19

Fractionation of Sample # 6*

(Set-up No. 4 - 20 mm. pressure)

Fraction	Vol. cc.	Per cent of whole	B.P.C. /20mm.	B.P.C. /760mm.	d_{25}^{25}	n_{20}^{20}	α_{25}^{25}
1	6.3	2.74	below 30**	165	0.8647	1.4681	+23.68°
2	4.0	1.74	58-59	171	0.8855	1.4650	-13.00°
3	9.5	4.13	62-63	175	0.8804	1.4641	-26.64°
4	16.2	7.04	82-83	206.5	0.9542	1.4787	+75.89°
5	24.0	10.43	83-85	208-209	0.9373	1.4711	+47.50°
6	33.0	14.35	86-87	211-212	0.9046	1.4570	+9.88°
7	5.7	2.48	91	213.5-214	0.9029	1.4618	+16.16°
8	66.8	29.04	95-97	218	0.9091	1.4689	+16.16°
9	25.0	10.87	97-101	228.5	0.9184	1.4681	-16.96°
Residue	25.0	10.87					
Total	216.8						

* This oil was distilled from the peppermint grown from Oregon root stock (See Table 1). Its physical and chemical constants differed widely from the Washington oils (See Table 9).

** Received in the cold-trap.

Refractionation of Pulegone Fractions:

The physical constants of the pulegone obtained from the various oils may be summarized as follows:

Table 20

Physical Constants of Regenerated Pulegone

Source	How Obtained	B.P. [°] /760mm.	d_{25}^{20}	n_D^{20}	α_D^{25}
Oil # 2	Fractionation followed by regeneration from sulfite-addition by steam distillation	220-223	0.9326	1.4840	+19.89°
Oil # 3	Fractionation		0.9332	1.4859	+23.30°
Oil # 4	(1) Regenerated from sulfite addition by steam distillation		0.9338	1.4858	+23.35°
	(2) Separated from sulfite-addition by addition of alkali only.		0.9181	1.4843	+20.65°
Oil # 7	Regenerated from sulfite-addition by steam distillation		0.9341	1.4868	+23.12°

In Table 21 the physical constants for pulegone, as reported from several sources are also summarized. The specific gravities and refractive indices as indicated in Table 20 show good agreement with the constants recorded therein. However with respect to the rotatory power it is to be observed that the figures recorded in Table 21 vary somewhat and the rotation as determined is more or less intermediate between the low and high figures cited.

Table 21
Recorded Physical Constants for Pulegone

	B.P.°C.	d	n_D	α_D
Grignard and Savard (106)	84 / 6mm.	$d_{\frac{15}{4}}^{\frac{15}{4}}$ 0.9346	n_D 1.4894	$[\alpha]_D^{20} +21.00^\circ$
Heilbron (110)	224	$d_{\frac{15}{4}}^{\frac{15}{4}}$ 0.9370	$n_D^{\frac{15}{4}}$ 1.4880	$[\alpha]_D^{20} +28.23^\circ$
Kon (111)	109 / 19mm.	$d_{\frac{15}{4}}^{\frac{15}{4}}$ 0.93808	$n_D^{\frac{15}{4}}$ 1.48810	
Schimmel and Co. (6)	224 / 760mm.	d_{15}^{15} 0.9405	$n_D^{\frac{15}{4}}$ 1.48796	$+20^\circ 48'$
Wallach (6)	221-223	d 0.9360	n 1.4846	

In order to determine if the optical rotations for the pulegone fractions as recorded in Table 20 could be altered by further fractionation some of these samples were re-fractionated. The results are given in Tables 22, 23, and 24.

Table 22

Refractionation of Pulegone From Oil # 3 (250 cc.)

(Set-up No. 4 - 20 mm. pressure)

Fraction	Vol. cc.	B.P.°C. /20mm.	B.P.°C. /760mm.	d_{20}^{20}	n_D^{20}	α_D^{20}
1	2	95	209.5	0.9162	1.4672	+59.64°
2	2	99	216	0.9211	1.4697	
3	4	99-101	220	0.9239	1.4727	+38.56°
4	4	101	222	0.9245	1.4786	+30.20°
5	8	102	217	0.9294	1.4836	+26.04°
6	4	102-103	222.5	0.9316	1.4849	+26.16°
7	6	104	222.5	0.9291	1.4811	+34.68°
8	18	105-106	223	0.9315	1.4853	+26.88°
9	87	105-106	224.5	0.9321	1.4861	+24.99°
10	75	106-107	222	0.9315	1.4870	+22.54°
Total 228						

Table 23

Refractionation of Pulegone from Oil # 4

(Set-up No. 4 - 20 mm. pressure)

Fraction	Vol. cc.	B.P.°C. /20mm.	B.P.°C. /760mm.	d_{20}^{20}	n_D^{20}	α_D^{20}
(a) Pulegone from Oil # 4 (Separated by alkali <u>only</u>) about 200 cc.						
1	4.4	96-98	215-218	0.9309	1.4847	+28.04°
2	3.6	99-101	220.5	0.9319	1.4861	+25.92°
3	122.0	101	222.0	0.9324	1.4866	+23.32°

Note: About 1.5 cc. recovered from cold-trap - d_{20}^{20} 0.9273,
 n_D^{20} 1.4680.

Table 23 (Continued)

Fraction	Vol. cc.	B.P.C. /20mm.	B.P.C. /760mm.	d_{25}^{25}	n_D^{20}	α_D^{25}
(b) Pulegone regenerated by steam distillation - about 270 cc.						
1	4	87-88	220.5	0.9303	1.4773	+47.00°
2	9	96-97	222	0.9299	1.4800	+38.24°
3	23	99-101	222	0.9297	1.4822	+32.08°
4	24.5	101	222	0.9313	1.4850	+26.16°

Note: About 2 cc. recovered from cold-trap-- d_{25}^{25} 0.9124, n_D^{20} 1.4491. In (a) and (b) in the above table the fractionation was discontinued when the temperature reached a steady 101°C.

Table 24
Refractionation of
Pulegone from Oil # 7 (about 360 cc.)

Fraction	Vol. cc.	B.P.C. /20mm.	B.P.C. /760mm.	d_{25}^{25}	n_D^{20}	α_D^{25}
1	1.5	*	168-169	0.9121	1.4480	
2	8	93-96	209.5	0.9226	1.4709	+50.38°
3	27	101-102	222.5	0.9312	1.4850	+25.97°
4	84	103	223.5	0.9323	1.4870	+24.56°

* Received in cold-trap.

Note: The fractionation was discontinued when the temperature reached a steady 103°C.

DISCUSSION OF RESULTS OF FRACTIONATION

The abnormal properties of the oils originally observed by Hiner have been substantiated by fractionation with several different columns of varying efficiency. A contemporary oil of different origin (i.e. Oregon root stock) has also been fractionated and found to stand intermediate between the Washington oils and the official standards.

In particular, the high ketone (presumably pulegone) content of the Washington oils, as reported by Hiner and as indicated by the physical constants (See Table 9) and the sulfite assays (See Table 12) has been verified in each instance. When purified through bisulfite addition and regeneration these ketone fractions were found to have the following average characters: b.p. 220-223 °C./760 mm., d_{20}^{20} 0.9329, n_D^{20} 1.4850, $n_D^{20} + 21.6$. These figures are in good agreement with those reported for pulegone by several authors (See Table 21).

No evidence was found during fractionation of the presence in appreciable amount of a ketone incapable of reacting with sulfite or bisulfite (e.g. menthone).

In the fractionation of oils # 2 and # 3, although 500 cc. was used in each case, no fraction corresponding to a menthol fraction was obtained. When the oil unabsorbed by bisulfite, representing a total of 1200 cc. of oils # 3, # 4, and #7, was examined fractions 3, 4, and 5 (totaling 14 cc.)

were found to assay 14.04, 44.1 and 39.93 % free alcohol (calculated as $C_{10}H_{20}O$). These fractions combined represent just over 1 % of the original samples used. Thus the content of free menthol in these oils, reported by Hiner as ranging from 7.23 - 15.15 %, could not be substantiated by fractionation. Even the lower values, 2.20 - 10.08 %, obtained by the Brignall method (See Table 10) could not be approached.

The terpene content of the various Washington oils as indicated by fractionation was always low. In no case was the combined distillate boiling in the terpene range more than 5 % of the original total taken.

The fractionation studies were of little value in determining the possibility of the presence of appreciable amounts of esters in the Washington oils. Apparently the ketone present in such high proportion was responsible for considerable polymerization during distillation. As a result the residue became so thick that it became impractical to continue the process at the higher temperatures.

Oil # 6 (from peppermint grown from Oregon root stock) was found to possess quite different distillation characteristics from the Washington oils. This was to be expected having consideration for its relatively high menthol content, 26.08 % found by the Brignall method (See Table 10). The lower boiling fractions had a distinct odor of cineol and well defined menthone and menthol fractions were obtained

at the expected levels. The unusual features of this oil, as revealed by fractionation, appear to be the dextro-rotatory power of the lowest boiling fraction and the α_D of $+75.89^\circ$ observed for fraction No. 4. This latter seemed to have been carried over to some degree into the succeeding menthone fractions with the result that plus rotations were still obtained. The unexpectedly high specific gravity of 0.9542 obtained for fraction No. 4 also seemed to be significant. This along with the high rotation suggested a similarity to the new constituent reported by Carles (79) and which Wienhaus and Dewein (80) identified as 3,6-dimethylcoumarone tetrahydride (See Part II, Chapter 1). The menthol fractions, 8 and 9, although l-rotatory, do not possess this power to the degree to be expected. There are at least two possibilities which may be advanced by way of explanation: (1) The oil when assayed by the sulfite method showed 3% ketone (See Table 12); this may be pulegone and may contaminate the menthol fractions. (2) Isomeric menthols, especially d-neomenthol which has been reported (6) as a constituent of Japanese peppermint oil, may be present. This latter possibility is strengthened by the suggestion of Pickard and Littlebury (101) that l-menthone is first formed in the plant but is afterwards reduced to a mixture of l-menthol and d-neomenthol.

The re-fractionation of seemingly pure pulegone fractions with the relatively efficient column used in set-up

No. 4 has brought to light an interesting fact. Regardless of whether the pulegone was originally obtained by fractional distillation or by separation through bisulfite addition and regeneration, the first sub-fractions possessed a considerably higher dextro-rotatory power than did the original ketone. This may be explained by either; (1) the presence of some constituent boiling so close to pulegone that a column of high efficiency is necessary to disclose it, or (2) to chemical change as a result of the application of heat or bisulfite. The evidence brought out in Table 23 lends some support to the theory that heat may have been a factor. In this case where the ketone was recovered from its bisulfite addition compound without resorting to steam distillation, the highest dextro-rotation recorded during fractionation was $+28.04^{\circ}$. This is to be compared with $+47.00^{\circ}$ observed for the first fraction when steam distillation was employed. The possibility of chemical change as a result of bisulfite treatment is mentioned because all of the samples of pulegone examined in this manner, whether originally obtained by fractionation or not, were at one time or another reacted with bisulfite prior to refractionation.

A comparison of the properties of the pulegone sub-fraction found to have the highest rotation ($+59.64^{\circ}$) with those of fraction 4 (Oil # 6) is of interest; and is presented in Table 25.

Table 25

Comparison of Pulegone Fraction (+59.64°) with Fraction 4
(Oil # 6)

Fraction	B.P.C. /20mm.	B.P.C. /760mm.	d_{25}^{25}	n_{20}^D	α_{25}^{25}
1 (Pulegone from Oil # 3)	95	209.5	0.9162	1.4672	+ 59.64°
4 (Oil # 6)	82-83	206.5	0.9542	1.4787	+75.89°

The considerable difference in the specific gravities observed in these two instances suggests that different constituents may be involved. It is necessary to keep in mind also the fact that the pulegone was reacted with bisulfite and recovered from the addition product prior to refractionation. This detracts from the possibility that 3,6-dimethylcoumarone tetrahydride may also be responsible for the high dextro-rotatory power in this instance as it is suspected to be in oil # 6.

Chapter 4

IDENTIFICATION OF CONSTITUENTS

I. Low-Boiling Constituents:

In Chapter 3 of this section it was pointed out that the terpene content in these oils, as revealed by fractionation, was relatively small. The results of the fractionation studies in this connection are summarized in Table 26.

Table 26

Data on Terpene Fractions (after Re-fractionation)

Fraction	Vol. cc.	B.P.C. /760mm.	d_{25}^{25}	n_{20}^{20}	α_{25}^{25}
Oil # 2 (500 cc.)					
2 I	4	155.4	0.8548	1.4659	-16.0°
2 II	5	169	0.8413	1.4712	-48.8°

Oil # 3 (500 cc.)					
1	3.4	156-161	0.8581	1.4680	-26.8°
2 I	2.5	157	0.8556	1.4690	-16.8°
2 II	7.5	168-170	0.8402	1.4706	-55.2°

Oil # 3, 4, 7 (1200 cc.)					
1	4	168.5	0.8467	1.4679	-47.04°
2	4	171	0.8399	1.4675	-68.84°

Oil # 6 (Oregon) (230 cc.)					
1	6.3	165	0.8647	1.4681	+23.68°
2	4.0	171	0.8855	1.4650	-13.00°
3	9.5	175	0.8804	1.4641	-26.64°

Identification of Terpenes:

The results insofar as identification of terpenes is concerned are inconclusive. The small amount of each fraction that was available prevented an extensive examination of it.

1. α -pinene: The recorded constants for α -pinene vary somewhat. Gildemeister and Hoffmann (6) give for 1- α -pinene: b. p. 153.5 - 154.5 °C.; d_{4}^{20} 0.8626; n_{D}^{20} 1.4690; $[\alpha]_D^{20}$ -43.8°. Heilbron gives: b. p. 155-156 °C.; d_{4}^{20} 0.8595; n_{D}^{20} 1.47299; $[\alpha]_D^{20}$ -47.2°.

Two of the fractions, fraction 2 I (Oil # 2) and fraction 2 I (Oil # 3), whose constants checked reasonably well with the above, were converted into their nitrosochlorides. The product, after recrystallization from chloroform and methyl alcohol, melted 103 - 105 °C. A crystalline nitrol-piperidide (m. p. 121 - 122 °C.) was prepared in the one instance but an attempt to prepare the nitrol-benzylamine in the other was unsuccessful.

Regarding the nitrosochloride of α -pinene, Gildemeister and Hoffmann (6) state: "The melting-point of the recrystallized compound is 103 °C., though melting-points as high as 115 C. have been observed. . . . The melting-point of the pinene nitrol-piperidine is 118 - 119 °C." It seems likely that these remarks refer to the inactive form of α -pinene, since the melting-point of the optically active pinene nitrosochlorides is given by Heilbron and others as 81-81.5 °C.

The optical rotation of only -16° (approximately) observed for these fractions would indicate the inactive form of α -pinene to be present.

2. 1-Limonene: The boiling-points, specific gravities and refractive indices of fractions 2 II (Oil # 2), 2 II (Oil # 3) and 2 (# 3, 4, and 7 mixture) are within or close to those recorded for limonene, its inactive form (dipentene) and phellandrene. The method of Parry (7) for the preparation of a tetrabromide was followed in each instance but without obtaining a derivative, even though special precautions were taken to keep the mixture at a low temperature during the addition of the bromine. When applied to a sample of d-limonene (from orange oil) this method produced a good yield of crystalline tetrabromide.

3. 1-Phellandrene: Two methods, differing somewhat in detail, were tried for the preparation of a nitrosite. However each of the fractions mentioned in the preceding paragraph failed to yield this derivative.

4. d-Carene: The dextro-rotatory fraction No. 1 from Oil # 6 was examined for d-carene which is stated (6) to boil at $165 - 169^{\circ}\text{C}$. The nitrosate appears to be the most satisfactory derivative for the characterization of this terpene. The method described by Simonsen (112) was used but no crystalline material was obtained.

Identification of Cineol:

The low-boiling fractions from the # 3, 4, and 7

mixture and more particularly from Oil # 6 smelled strongly of cineol. Consequently these fractions were assayed by the resorcin method (6). The results are indicated in Table 27.

Table 27
Cineol Determinations for Low-boiling Fractions

Fraction	Volume Used cc.	Volume Unabsorbed cc.	Per Cent Cineol
Oil # 3, 4, and 7*			
1	1	0.74	26
2			

Oil # 6			
1	2	1.86	7
2	2	0.86	57
3	2	1.18	41

* A small amount obtained in the cold-trap during fractionation assayed 35 % cineol.

The resorcin mixture after being freed from unabsorbed oil was treated with solution of KOH and steam distilled. In this manner 0.5 - 1 cc. of a colorless oil was recovered. It smelled strongly of cineol and its b. p. was determined at 171 - 172°C. /760 mm. and n_{D}^{20} 1.4588 (recorded (106) for cineol b. p. 174.4°C. /760 mm., n_{D}^{20} 1.45839. The quantity available did not permit the preparation of a derivative.

Identification of 1,3-methylcyclohexanone:

During the fractionation of the pulegone from oil # 7 (See Table 24) a small amount of oil (about 1 cc.) was caught in the cold-trap. Its characters were determined as follows: b. p. 168 - 169 °C. /760 mm.; d_{44}^{20} 0.9121 (approximately); n_D^{20} 1.4480. The low refractive index and a characteristic odor caused 1,3-methylcyclohexanone to be suspected. Wallach* records the following constants for pure d-methyl-1 cyclohexanone -3: b. p. 169 °C.; d_4^{20} 0.909; n_D 1.4460; $\alpha_D +12.5^\circ$.

Preparation of Semicarbazone: The whole amount available was involved in reaction with semicarbazide in the usual manner. A crystalline substance, m. p. 178 - 179 °C. was obtained. (Wallach* gives 180 °C.) The quantity was insufficient for further purification.

II. Menthone and Free Menthol

A. Washington Oils.

With respect to the Washington oils, it has already been pointed out that the only fractions suggestive of containing menthone or free menthol were obtained by fractionation of the residue uncombined with bisulfite from the mixture of oils # 3, 4 and 7. (see Table 18). In this instance fractions 3, 4 and 5 possessed a similar penetrating, lingering odor suggestive of menthol but partly masked

* Wallach, O., Terpene and Camphor (2nd. ed.) p. 420.

by a sweetish, though somewhat musty smell. By the Brignall method their respective free alcohol contents, calculated as menthol, were 14.04, 44.1, and 39.93 per cent.

Identification of Menthone:

The constants recorded in Table 18 for fraction 3 are as follows: b. p. 201°C. /760 mm.; d_{4}^{20} 0.8893; n_D^{20} 1.4559; $\alpha_D^{20} +10.4^\circ$. Constants for menthone regenerated from its semicarbazone are recorded (6) as follows: b. p. 208°C.; d 0.8940; n_D 1.4496.

A semicarbazone was prepared from this fraction which, after one recrystallation melted 181 - 182°C. Gildemeister and Hoffmann (6) give 184°C. for menthone semicarbazone, while Simonsen (106) gives 185 - 186°C.

Thus the presence of a very small amount of menthone in this mixture of Washington oils is considered to have been established. However, the observed rotation of $+10.4^\circ$ differs considerably from the reported (6) $-20^\circ 27'$ to $-26^\circ 10'$. The most likely explanations of this dextro-rotation are that either, (1) isomeric menthols make up all or part of the alcohol content of this fraction, or (2) some other unknown factor is present, possibly d-isomenthone, $[\alpha]_D^{25} +95$).

Identification of Menthol:

1. Preparation of phenylurethane: Phenylurethanes were prepared from fractions 4 and 5 by allowing pheryl isocyanate in slightly more than the calculated amount to

react with the oil in the cold. With fraction 5 the reaction mixture formed a solid mass which after suction filtration was recrystallized from boiling pentane and obtained in spear-shaped masses of slender crystals, m. p. 111 - 112°C. The reaction mixture produced a further yield of solid material which after recrystallization as above produced only short translucent rods, m. p. 107 - 108°C. With fraction 4 a much smaller initial yield of phenylurethane was obtained. When recrystallized it appeared to be more soluble in pentane and was only obtained after removal of the greater part of the solvent. Its melting point was also 107 - 108°C.

Several authors give the melting point of menthyl phenylurethane as 111 - 112°C. It is to be noted also that Gildemeister and Hoffmann (6) cite 107 - 108°C. as the melting point of d-neomenthol phenylurethane.

2. Preparation of 3,5-dinitrobenzoate: This ester was prepared from fraction 5, by the method given by Shriner and Fuson (8). A good yield was obtained which, after recrystallization from about 80% alcohol, melted 152 - 153°C. Cohen and Arnes (113) cite 153 - 154°C. for menthyl 3,5-dinitrobenzoate.

3. Preparation of hydrogen phthalate esters: The melting-points of the hydrogen phthalate esters appear to provide better opportunity for clear distinction between the various menthol isomers. However, using the method as outlined on p. 24, only a viscous residue was obtained with

fraction 4. Efforts to get it into a crystalline form were unsuccessful even though several different solvents were tried.

B. Oil # 6 (Oregon).

This oil, as indicated in Table 19, differed widely from the Washington oils with respect to these intermediate fractions. Fractions with physical constants suggesting menthone and menthol were obtained at the expected levels.

Identification of Menthone:

The two possible menthone fractions appeared to be:

Fraction 6. 14.35 % of sample; b. p. 211 - 212°C.

/760 mm.; d_{20}^{20} 0.9046; n_D^{20} 1.4570; α_D^{20} +9.88°

Fraction 7. 2.48 % of sample; b. p. 213 - 214°C.

/760 mm.; d_{20}^{20} 0.9029; n_D^{20} 1.4618; α_D^{20} +16.16°

A semicarbazone was prepared from fraction 6, which in its impure form melted at 180°C. After digestion with pentane and with ether and recrystallization from methyl alcohol this was raised to 187°C.

A similar semicarbazone was prepared from fraction 7, m. p. 187°C. It is to be noted also (see below) that fractions 4 and 5 both gave small yields of semicarbazone melting at 187°C.

When these semicarbazones were mixed and hydrolyzed by means of steam distillation with oxalic acid a small amount of a light yellow oil was obtained. It had a menthol-like

odor and the following constants were found: b. p. 209 - 210 °C. /760 mm.; d_{4}^{20} 0.8900; n_D^{20} 1.4509; α_D^{20} -13.76 (approximately).

Thus it appears that menthone is a constituent of Oil # 6 and in approximately normal amounts.

Identification of Menthol:

The two possible menthol fractions from this oil appeared to be:

Fraction 8. 29 % of sample; b. p. 218 °C. /760 mm.;

d_{4}^{20} 0.9091; n_D^{20} 1.4689; α_D^{20} -16.16°

Fraction 9. 10.87 % of sample; b. p. 228.5 °C.

/760 mm.; d_{4}^{25} 0.9184; n_D^{25} 1.4681; α_D^{25} -16.96°

1. Separation of menthol: After prolonged treatment in a cooling bath, fraction 8 yielded a total of approximately 25 gm. of needle-shaped crystals which re-melted at room temperature (25 - 30 °C.). The liquid so obtained had the following constants: d_{4}^{25} 0.9013; n_D^{25} 1.4631; α_D^{25} -29.03°. The mother liquor, after separation of the solid, gave d_{4}^{25} 0.9080; n_D^{25} 1.4671; α_D^{25} -7.53°. Simonsen (106) records for l-menthol: b. p. 216 °C. /760 mm.; d_{4}^{15} 0.9040; n_D^{20} 1.46096; $[\alpha]_D^{20}$ -49.44°.

The mother liquor still showed 59.07 % free menthol by the Brignall method. R. E. Kremers (114) reports that removal of menthol by freezing out is a matter of considerable difficulty. He was able to remove only about 12 % in this

manner. Gordon (115), likewise, could only obtain 13 %. The latter expresses the view that a common mechanism hinders the crystallization of menthol.

2. Preparation of Phenylurethane: The liquid obtained by melting the separated crystals was treated with phenyl isocyanate. The resulting phenylurethane, after recrystallization, was obtained as a mass of fine white crystals, m. p. 111°C . (recorded for menthol $111 - 112^{\circ}\text{C}$.).

3. Preparation of α -Naphthylurethane: By a similar procedure an α -naphthylurethane was obtained, m. p. $117 - 119^{\circ}\text{C}$. Bickel and French (116) record 119°C . for menthyl α -naphthylurethane.

4. Preparation of 3,5-dinitrobenzoate: The ester, after recrystallization, was obtained as a mass of soft, white, glistening crystals, m. p. 153°C . (recorded for menthyl 3,5-dinitrobenzoate, $153 - 154^{\circ}\text{C}$.).

Identification of 3,6-Dimethylcoumarone Tetrahydride (Menthofurane)

Fractions 4 and 5 obtained during the fractionation of Oil # 6 (see Table 19) have boiling points almost within the menthone-menthol range. However, in view of the rotations observed for these fractions, they must be considered as definitely abnormal from the standpoint of the usual constituents of peppermint oils. The constants observed for these fractions are as follows:

Fraction 4. 7.04 % of sample; b. p. 206.5°C .

/760 mm.; d_{25}^{25} 0.9542; n_D^{25} 1.4787; $\alpha_D^{25} + 75.89^\circ$.

Fraction 5. 10.43 % of sample; b. p. 208 -

209°C. /760 mm.; d_{25}^{25} 0.9373; n_D^{25} 1.4711; $\alpha_D^{25} + 47.50^\circ$.

The similarity of these characters to those of the constituent reported by Carles (79) and identified by Wienhaus and Dewein (80) has already been pointed out.

Fraction 5 gave a fairly good yield of a semicarbazone, m. p. 187°C. Likewise a mixture of the two fractions, after an unsuccessful attempt to refractionate it, reacted with semicarbazide to yield a semicarbazone which melted at 187°C. The unreacted oil was recovered from this reaction mixture as a thick liquid, light brown in color. Its characters were determined as follows: d_{25}^{25} 0.9999; n_D^{25} 1.4908; $\alpha_D^{25} + 64.6^\circ$.

It therefore appeared that these fractions consisted of some menthone along with a proportionally greater amount of a constituent possessing a high degree of dextro-rotatory power.

It was noted the liquid was inclined to creep up the wall of the test tube and to form small colorless crystals thereon. There was also some evidence of a greenish color on the glass. These crystals were found to melt at 183-185°C. A small yield was also obtained when 2 cc. of the fraction was oxidized with chromic acid. Wienhaus and Dewein (80) state that the substance 3,5-dimethylcoumarone

tetrahydride is oxidized in air to a colorless acid melting at 186°C.

III. Proof of Pulegone:

The physical constants of the well-defined pulegone fractions obtained from each of the Washington oils have been summarized in Table 20. After purifying the ketone through bisulfite addition and regeneration, the characters were re-determined and may be recorded in the following average values: b. p. 220 - 223°C. /760 mm.;

d_{25}^{25} 0.9329; n_D^{20} 1.4850; α_D^{25} + 21.6°. Schimmel and Co. (6) record for pulegone (see Table 21); b. p. 224°C. /750 mm.;

d_{15}^{15} 0.9405; n_D^{20} 1.48796; α_D + 20°48'.

1. Treatment with Semicarbazide: When reacted with semicarbazide under the conditions outlined above (see p. 19), good yields of semicarbazones were obtained from all pulegone fractions. After standing several hours the separated crystalline material was removed by suction filtration and the mixture successively diluted for several days. In this manner several separations were obtained, the final ones being less crystalline and more granular in appearance. There was also a decline in melting-point the granular residues in some cases melting as low as 120°C. The impure semicarbazone (m. p. 158 - 166°C.) after recrystallization from alcohol was obtained as small white crystals, melting-point 169 - 171°C. Further recrystallizations from this solvent

failed to raise this appreciably. However, when the procedure of Hugh, Kon, and Linstead (25) was applied the crystals obtained were more translucent in appearance and melted $173 - 174^{\circ}\text{C}$. (Recorded for pulegone semicarbazone, Heilbron (110) 174°C ., Gildemeister and Hoffmann (6) $167.5 - 168^{\circ}\text{C}$.)

2. Preparation of 2,4-dinitrophenylhydrazone: The method of Brady (117) was used in the preparation of this derivative. The red granular substance so obtained was recrystallized from alcohol (95 %) and was then found to melt, rather indefinitely, at 142°C . After recrystallization from pentane it was obtained in dark-red crystalline plates, melting-point $147 - 148^{\circ}\text{C}$. Campbell (118) gives 147°C . for pulegone 2,4-dinitrophenylhydrazone while several other authors record 142°C .

3. Hydrolysis of Pulegone: 82 cc. of regenerated pulegone (from Oil # 2) was hydrolyzed by boiling with an equal volume of 85 % formic acid in an oil-bath for 16 hours, as described by Wallach (119). The reaction mixture was then neutralized with a concentrated potassium hydroxide solution, the oil separated in a separatory funnel and washed with water until the washings were free from alkali. After drying over anhydrous sodium sulfate, about 55 cc. of oil was recovered.

The dried oil was distilled at 20 mm. using set-up No. 4. A fraction, measuring 17 cc., was thus obtained for

which the following constants may be recorded: b. p. 60°C . / 20 mm., 163.5°C . / 760 mm.; d_{4}^{25} 0.9190; n_{D}^{20} 1.4456; $\alpha_{D}^{25} + 12.2^{\circ}$ (equivalent to $[\alpha]_{D} + 13.39^{\circ}$). The following constants have already been cited (see p. 113) for 1,3-methylcyclohexanone: b. p. 169°C .; d_{4}^{21} 0.9090; n_{D} 1.4460; $\alpha_{D} + 12.5^{\circ}$.

A semicarbazone was prepared which after recrystallization melted $179 - 180^{\circ}\text{C}$.

Thus 1,3-methylcyclohexanone, one of the hydrolysis products of pulegone has been identified.

Identification of pulegone in Oil # 6 (Oregon): This oil was found (see Table 12) to contain 3 % of some ketone other than menthone (i.e. capable of combining with bisulfite). Thus pulegone was suspected and 20 cc. of fraction No. 9 was treated with semicarbazide in the usual manner. About 5.5 gm. of impure semicarbazone was obtained. After purification and recrystallization a crystalline product resembling pulegone semicarbazone was obtained, m. p. $171 - 172^{\circ}\text{C}$.

IV. Identification of d-Isomenthone:

Two observations made when reacting the various pulegone fractions with semicarbazide seemed to warrant further study. Stated briefly they were as follows: (1) some other substance of relatively high melting-point was always present in the first separation of pulegone semicarbazone;

(2) excessive dilution of the reaction mixture after the removal of all, or the greater part of the pulegone semicarbazone, always resulted in a portion of granular white material, melting indefinitely between 125° and 150° C.

It was at first believed that the high-melting substance which was almost entirely insoluble in water, was hydrazodicarbonamide (m. p. $245 - 246^{\circ}$ C.). However it seemed unlikely that the amount of heat used in the reaction was sufficient to account for such amounts of this material as were sometimes obtained. Furthermore, repeated washing with pentane and ether followed by digestion with boiling alcohol, usually left a residue which melted $256 - 260^{\circ}$ C. It seemed, therefore, that some other explanation must be sought.

Another possibility seemed to be that a pulegone semicarbazide-semicarbazone was being formed through the addition of one molecule of semicarbazide to the semicarbazone at the double bond. Such compounds are known for some unsaturated ketones but Busse and Gurewitsch (120) state that attempts to obtain them from compounds with conjugated double bonds, of the pulegone type, have been unsuccessful (several references to this effect are cited).

It was then noted that Barrowcliffe (121) while investigating the constituents of essential oil of American Pennyroyal (Hedeoma Pulegioides (L.) Pers.) reported circumstances similar to those noted above. From a 10 gm.

fraction (b. p. 212 - 217°C.) from which the pulegone had been removed as completely as possible by bisulfite addition, he obtained a semicarbazone, the greater part of which was insoluble in hot absolute alcohol. After separating out some l-menthone semicarbazone the mother liquor yielded what is described as "3.5 gm. of a substance melting 136 - 139°C., which crystallized in hard masses of imperfectly formed cubes.the mother liquors also yielded about 3 gm. of uncrystallizable oil" After determining the properties of the ketone regenerated from this material, Barrowcliffe concluded d-isomenthone to be a constituent of the oil under examination.

Barrowcliffe does not appear to have connected this low-melting semicarbazone with the material insoluble in hot absolute alcohol. It should be noted, however, that Heilbron (110) reports a melting-point of 264°C. for d-isomenthone semicarbazone.

When all available pulegone fractions having a α_D of +38° or more (see Tables 22, 23, 24), were mixed and reacted with semicarbazide in the usual manner, a white solid separated which, after digesting with pentane and with ether (in which it seemed to be partially soluble) was obtained in hard chalk-like masses and melted indefinitely 135 - 142°C. It did not crystallize well from alcohol but had a tendency to form doughnut-shaped masses from methyl alcohol (m. p. 140-142°C.). A small amount of uncrystallizable oil was also

obtained which, after long standing set into a white mass similar to the above. It was not available in sufficient amount for regeneration of the ketone.

On two occasions low-melting solid material recovered from pulegone-semicarbazide reaction mixtures after several recrystallizations also gave small yields of stellated translucent crystals, melting-point 141 - 142°C. In this connection it is of interest to note that R. E. Kremers (103) after treating a pulegone fraction obtained from oil of Mentha canadensis with semicarbazide records a similar result. He did not identify the ketone responsible.

V. Examination of High-Boiling Residues:

A. Washington Oils.

The examination of the still residues boiling above the pulegone range (220 - 223°C.) has been restricted by two circumstances:

- (1) The relatively small amounts of such residues available.
- (2) The presence of much resinous material as a result of continued heating of the pulegone present in the oil.

Although ester contents ranging from 3.66 to 4.67 % (calculated as menthyl acetate) were reported for the various Washington oils (see Table 9), it was subsequently

shown that pulegone itself was capable of yielding an average ester value of 7.21 (= 2.54 % combined alcohol, calculated as menthyl acetate).

Thus, from a study of physical and chemical constants alone, the amount of combined alcohols present in these oils is apparently very small. This was borne out by the fractionation studies. When the pulegone fraction had been obtained the residue in the still became so thick that further distillation soon became impractical.

Saponification of the various still residues produced very little in the way of results. The method followed was to heat the dark colored residue with an excess of N/2 alcoholic KOH from 1 to 2 hours. The greater part of the alcohol was evaporated off and the mixture freely diluted with water and extracted several times with ether. The combined ether extracts were then washed with successive portions of water until free from alkali. The ether was removed by distillation leaving a dark semi-solid residue.

The results of attempts to fractionate such residues are indicated in Table 23. Because of the small amounts involved a small distillation flask with a short built-in Vigreux column was employed. The resinous matter present proved a considerable obstacle in this operation.

Table 28

Fractionation of Saponification Residues (Washington Oils)

Fraction	Volume cc.	B.P.C.	d_{25}^{25}	n_D^{20}	α_D^{25}
Oil # 2 (1 cc. approximately)					
No attempt at fractionation.					

Oil # 3 (7.5 cc. approximately)					
1	0.5	96 / 5mm.			
2	2.5	96-106 / 5mm.	0.9315	1.5057	
Residue	1.5				

Oil # 3, 4, and 7 (see Table 17)					
1	0.9	184-186 / 760mm.		1.4680	
2	2.6	234 / 760mm.	0.9289	1.5030	-5.16°
Residue					

In each instance the saponified residue, prior to fractionation had an unfamiliar sweetish, though somewhat musty smell. After fractionation this smell was still evident in the fractions although masked by an odor of decomposition.

B. Oil # 6 (Oregon).

In this case the residue from fractionation, amounting to about 25 cc. (see Table 19) was saponified as outlined above. Since the original oil which assayed 10.94 % ester (calculated as menthyl acetate) was relatively free from

pulegone, less difficulty was encountered with resinous compounds. The dark residue, after evaporation of the ether was transferred to the small distilling flask and heated in an oil bath at 760 mm. pressure. The results of the fractionation are shown in Table 29.

Table 29

Fractionation of Saponification Residue (Oil # 6)

Fraction	Vol. cc.	B.P.C. /760mm.	d_{25}^{25}	n_D^{20}	α_D^{25}	Per cent Menthol Content*
1	3.5	206-212	0.9020	1.4683	-22.36°	75.42
2	2.2	215-217	0.9071	1.4769	-16.40°	
3	3.6	228.5	0.9356	1.4900	+10.80°	26.60
Residue						

* Brignall method.

Although the odor of menthol was more pronounced in these fractions than in the case of the Washington oils, the peculiar sweetish odor still prevailed.

Identification of Menthol:

1. Phenylurethane: This compound prepared from fraction 1, after recrystallization from boiling pentane, was obtained as a fleecy, white mass of crystals. The melting-point was 112°C. (recorded for menthyl phenylurethane, 111 - 112°C.).

2. 3,5-dinitrobenzoate: The ester, also prepared

from fraction 1, crystallized from about 80 % alcohol in long slender needles, m. p. 154°C . (recorded for menthyl 3,5-dinitrobenzoate, $153 - 154^{\circ}\text{C}$.).

VI. Volatile Acids:

Because of the ester content of these oils, the total amount of volatile acid available from any one sample was too small for a satisfactory investigation. However the aqueous-alkaline saponification mixtures, after extraction with ether, were concentrated, acidified with dilute H_2SO_4 and distilled as long as the distillate remained acid.

The amount of approximately $\text{N}/10 \text{ Ba(OH)}_2$ required to neutralize the acid distillates so obtained was 14 cc. in the case of Oil # 3, 15 cc. for the # 3, 4, and 7 mixture, and 29 cc. for Oil # 6.

It is believed that the Duclaux distillation method, as elaborated by Gillespie and Walters (27) is capable of successful adaptation to the identification of volatile acids from such saponification residues. In this study circumstances only allowed it be applied to the acid distillate obtained after the saponification of the residue from Oil # 3. The results are inconclusive. When calculated for a mixture of acetic and isovaleric acids, the indications were that the mixture did not consist only of these two acids. Due to the small amount of total acid involved the results were not considered sufficiently accurate for further calculations.

Discussion of Results:

The only terpene identified as a constituent of these oils is α -pinene. The melting point of the nitrosochloride and the nitrol-piperidide indicate the inactive form of the terpene, possibly in the presence of some 1- α -pinene since the rotation of the fractions was approximately -16° . The odor of the terpene fractions boiling $168 - 175^{\circ}\text{C}$. did not suggest limonene and no tetrabromide could be obtained.

Menthol has been identified as a constituent of the Washington oils, in which it was apparently present only in traces, and of the Oregon oil, in which a value of approximately 25 per cent free alcohol was determined by assay. In both cases, particularly in the saponification residues, there was a strong odor suggestive of d-neomenthol. The identification of this constituent is not considered to be conclusive since crystalline hydrogen phthalate esters were not obtained for characterization. The melting-point of $107 - 108^{\circ}\text{C}$. reported (6) for the phenylurethane of d-neomenthol is rather close to that of menthyl phenylurethane for a clear-cut distinction. However the crystal formation and solubility in pentane of the phenylurethane having that melting-point appeared to differ from those of the menthyl phenylurethane obtained.

Menthone, likewise has been found in traces in the Washington oils and in considerable proportions in Oil # 6.

Its identity was established through its semicarbazone, m. p. 184 - 187°C. and by the physical constants of the ketone regenerated from semicarbazone.

3,6-Dimethylcoumarone tetrahydride (Menthofurane), boiling fractionally lower than menthone, also has been separated from Oil # 6 (Oregon) and identified. The high degree of efficiency of column used in Set-up No. 4 in a partial separation of this constituent from the menthone. Its identity was established through a consideration of its physical constants compared to those reported by Charles (79) and its oxidation product, as reported by Wienhaus and Dewein (80).

Pulegone, a major constituent of all the Washington oils has been identified by the preparation of a semicarbazone and a 2,4-dinitrophenylhydrazone. It was also proven by hydrolysis with the resulting formation of 1,3-methylcyclohexanone. Pulegone was also identified, through its semicarbazone, as a minor constituent of Oil # 6 (Oregon).

There appears to be sufficient evidence upon which to base an assertion that d-isomenthone was associated in small amounts with the pulegone in the Washington oils. The experimental evidence arises chiefly from the separation of semicarbazones, believed to be α - and β -forms of d-isomenthone semicarbazone from pulegone-semicarbazide reaction mixture. The form insoluble (in alcohol) melted 257 - 260°C. while the readily soluble form melted rather indefinitely, 135 - 142°C.

Heilbron (110) records 264°C . for d-isomenthone semicarbazone. Other workers (122) give $125 - 138^{\circ}\text{C}$. The latter state that the isolation of a homogeneous semicarbazone of this ketone proved too tedious and the hydrochloride of the oxime is suggested as a more practical derivative. However there was not sufficient material to permit regeneration of the ketone for further characterization. The α -form appeared to be more than usually stable and was not hydrolyzed by steam distillation with either oxalic or sulfuric acids.

The resinous material present in the residues remaining after fractionation of the Washington oils was found to interfere with the separation of the alcohols present in small amounts in the combined state. Menthol has been identified as a product of the saponification of the esters in Oil # 6 (Oregon). The odor of all saponification residues suggested the presence of traces of some alcohol other than l-menthol.

Only traces of volatile acids were obtained from saponification residues. Duclaux constants, indicated that at least a third acid was associated with acetic and isovaleric acids in one of the Washington oils.

SUMMARY

1. The physical and chemical constants of the following native volatile oils have been reported: Oil of Pycnothymus rigidus, Oil of Solidago rigida, Oil of Eri-geron canadensis, Oil of Heterotheca subaxillaris. As far as quantities permitted, the principal constituents have been identified.

2. Quantitative determinations of the oil present in the leaf of Illicium floridanum indicate a seasonal variation from 0.20 to 0.50 %. The increased yield was accompanied by a considerable increase in the specific gravity and refractive index of the oil. At the same time there is marked decline in ester content. The pleasant characteristic odor of this oil appeared to be associated with an alcohol (probably tertiary) in the fraction boiling just below 200 °C. This odor is considered to be sufficiently attractive to warrant further study and a survey of its commercial possibilities.

3. Several abnormal oils of peppermint produced during the years 1929 - 1932 inclusive have been studied. These oils were distilled from plants grown from root stock originally obtained from Washington, D. C. A contemporary oil from plants grown from Oregon root stock also has been examined.

The Washington oils have been proved to consist mainly

of pulegone with menthol and menthone present only in traces. The Oregon oil, although well below the official standard, contained a good proportion of menthol. It also contained a small amount of pulegone.

In connection with the quantitative determination of free menthol it has been established that with the Brignall method there is less tendency towards the formation of enol acetates when pulegone is present than there is with the U. S. P. XI procedure. The elimination of saponification after acetylation is also a factor in favor of the Brignall method when oils containing pulegone are being assayed.

The following constituents from among the twenty-two listed for American peppermint oil by Gildemeister and Hoffmann (6) have been identified in these oils: Inactive α -pinene, cineol, l-menthone, l-menthol (both free and combined), pulegone (and its hydrolysis product 1,3-methylcyclohexanone).

The presence of an appreciable amount of 3,6-dimethylcoumarone tetrahydride has been detected in the Oregon oil. This is a newer constituent first reported in 1929 as occurring in oil of peppermint. It was identified in 1934 and given the name menthofurane. It is characterized by a high degree of dextro-rotatory power and a rather unpleasant odor.

Small amounts of d-isomenthone have been found to be associated with pulegone in the Washington oils.

d-Neomenthol is thought to have been present in small amounts. The odor of the saponification residues in particular suggested this isomeric menthol but identification was not conclusive.

The possible biogenetic significance of the abnormal features of these oils is quite obscure. It has been noted that a group of workers who examined oils of peppermint produced in India reported constants that bear a very close relationship to the abnormal oils in this study. They mentioned that these oils were inferior in odor and flavor and resembled a mixture of peppermint and pennyroyal. It is rather likely that these oils, which were also stated to contain isomeric menthols, contained a high proportion of pulegone, as did the abnormal oils in this study. In view of the latitudes involved in these two instances, the question arises if temperature can be a factor in disturbing the biochemical mechanism which ordinarily results in the production of menthol with the result that pulegone is produced instead.

The presence of small amounts of d-isomenthone in oils in which l-menthone is far below normal is a point of interest. It is worthy of special note that pulegone and d-isomenthone are reported to have been found associated together

in the oil from the quite unrelated Hedeoma pulegioides (American Pennyroyal). The mechanism through which l-menthone may be inverted to d-isomenthone has been reviewed by several authors. However there does not appear to be a direct connection, between the deficiency of l-menthone and the presence of d-isomenthone on the one hand and the high pulegone content on the other.

The probably presence of d-neomenthol in oil of peppermint would appear to be in harmony with several recent observations suggesting that menthone may be first formed by the plant and afterwards gives rise to menthol by a process of reduction. When this reduction is carried out in the laboratory d-neomenthol is known to be one of the products, although l-menthol is usually formed in very much the larger proportion.

The occurrence of 3,6-dimethylcoumarone tetrahydride in appreciable amount in oil of peppermint must raise a number of interesting questions the elucidation of which must remain for the future.

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BIOGRAPHICAL NOTE

Arnold Whitney Matthews was born December 10, 1901 at Summerside, Prince Edward Island, Canada.

He received his B. Sc. in Pharmacy from the University of Alberta in 1921 and his M. Sc. from the same institution in 1926. In 1923 he left a position in wholesale pharmacy to accept a position as a lecturer in the School of Pharmacy, University of Alberta, where he now holds the rank of Associate Professor of Pharmacy.

He first attended the University of Florida in the summer of 1930 and enrolled for the summer session in the School of Pharmacy, Purdue University in 1938.

In 1938 - 1939, having been granted sabbatical leave he entered the Graduate School at the University of Florida.

During his undergraduate career he helped to found and was the first President of the Pharmacy Club. He is a registered pharmacist, a member of the Alberta and Canadian Pharmaceutical Associations, and an associate-editor of the Canadian Pharmaceutical Journal. He is a member of Rho Chi.

This dissertation was prepared under the direction of the Chairman of the candidate's Supervisory Committee and has been approved by all members of the Committee. It was submitted to the Graduate Council and was approved as partial fulfilment of the requirements for the degree of Doctor of Philosophy .

Date July 26, 1941

J. M. Simpson
Dean

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